No. 35.

Second Edition.

(New Series.)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

BLACK-WATER FEVER.

BY

CAPTAIN S. R. CHRISTOPHERS, M.B., I.M.S.

AND

Dr. C. A. BENTLEY.

(Officers on Special Duty).

BEING THE FIRST REPORT TO THE ADVISORY COMMITTEE APPOINTED BY THE GOVERNMENT OF INDIA TO CONDUCT AN ENQUIRY REGARDING BLACK-WATER AND OTHER FEVERS PREVALENT IN THE DUARS.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA.

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SYNOPSIS OF CONTENTS.

PART I.

INTRODUCTORY.

PART II.

BLACK-WATER FEVER MALARIAL IN ORIGIN.

PART III.

BLACK-WATER FEVER DUE TO THE ACTION OF A SPECIAL HÆMOLYSIN.

PART IV.

NATURE OF THE HÆMOLYSIN IN BLACK-WATER FEVER.

PART V.

PART VI.

Appendix.

BLACK-WATER FEVER.

PART I.

INTRODUCTORY.

CHAPTER I.

Historical and General.

DISCUSSION of the ætiology and nature of Black-water Fever has occupied a host of authors, and a minute study of all that has been written on the subject would serve no useful purpose. It will be sufficient briefly to marshal all the known facts; to indicate the different views that have been held as to the nature of Black-water Fever; and to see what are the main issues now open for research.

Broadly speaking four main theories have been held as to the nature and origin of Black-water Fever; and around these different views can be grouped all the recorded facts.

These four theories are:—

I.—THAT BLACK-WATER FEVER IS A PERNICIOUS FORM OF MALARIAL ATTACK.

The early French observers (1) who first differentiated the disease from among the other pernicious fevers, and determined that its characteristic sign is hæmoglobinuria, not as had been previously thought the presence of blood or even bile in the urine, considered as a result of their clinical observations and the study of the distribution, that Black-water Fever is malarial in nature, evidently regarding it as a particular form of pernicious access. The American writers (2), who shortly after described the disease in the United States, did not even question the relation of Black-water Fever to malaria; but called the condition "malarial hæmaturia." F. (3) and A. Plehn (4), who besides clinical observation made examinations of the blood for malarial parasites, also arrived at the conclusion that Black-water Fever is some form of malarial attack.

A new phase in the study of the disease was introduced by the researches of several physicians in Greece (5) and of Tomaselli (6) in Sicily, who recorded cases of hæmoglobinuria closely resembling the hæmoglobinuric

fever described by the French and Americans from the tropics. In these researches the part played by malaria is not emphasized, the point that is incontestably proven being the action of quinine in producing these attacks. Nevertheless it is stated that the phenomenon is seen in malarial subjects, and the belief that malaria is in some way concerned is clearly present; Tomaselli definitely states that he considers a predisposition the result of chronic malarial infection to be necessary before quinine can have this action.

Some later researches of Koch (7) show the same action of quinine in association with the hæmoglobinuria of East Africa; but Koch is especially concerned that this condition is not malaria.

The idea then held was, it should be remembered, that a paroxysm of Black-water Fever is a pernicious form of malarial attack; and even at the present time there is some confusion on this point, writers sometimes pointing out clinical differences between an attack of malaria and one of Black-water Fever, as an argument against the malarial origin of the latter.

Hence, when Koch finding few or no parasites in his cases comes to the conclusion that it is not malaria, we must interpret him as referring to the disease process itself, with which he was concerned at the moment, and not with the conditions which may have given rise to it. Koch's researches were the first to show definitely that Black-water Fever is, in no sense, an attack of malaria; though Grawitz and Kohlstoch (20) had as early as 1892 given up the use of quinine in the treatment of their cases, because on examination of the blood they could find no malarial parasites. Since Koch, almost all those who have studied the disease have come to the conclusion that Black-water Fever is a condition quite distinct from, and dependent on, a different morbid process to that concerned in a malarial attack.

II.—That Black-water Fever is Quinine Intoxication.

That quinine under certain circumstances can induce attacks of Black-water Fever is undoubted. It is usual, however, for Europeans in the tropics to take quinine when they feel the onset of fever; and it is to be expected that in very many cases of Black-water Fever there will be a history of previous quinine administration; but a large number of cases are recorded in which, time after time, a dose of quinine has been followed with clockwork regularity by the appearance of hæmoglobinuria.

Even under ordinary clinical conditions, it is the experience of most observers that attacks of Black-water Fever have in most cases been induced by quinine, the symptoms commencing within four or five hours of its administration. On

the other hand, a number of cases have been recorded in which the patient had not taken quinine prior to his attack.

A study of recorded cases seem to point to the existence of two different conditions under which this drug may act in the production of hæmoglobinuria.

In one class of case quinine may have even been frequently employed with benefit in the treatment of malaria; until perhaps one day, when a dose has been taken for what has been supposed to be an actual or impending attack of malarial fever, its administration is followed in the course of a few hours by the onset of hæmoglobinuria. In these cases it frequently happens that subsequent doses of quinine fail to produce a repetition of the symptoms; this is the condition met with in the majority of Black-water Fever cases.

In another category must be placed those much rarer cases where quinine even in minute doses is invariably followed by the onset of hæmoglobinuria.

The view that quinine alone is sufficient to bring on attacks of the disease, apart from an acquired predisposition, has been upheld by only a few observers. Without exception, even those who have laid the greatest stress upon the part played by this drug in the production of hæmoglobinuria, have taken care to qualify their statements by pointing out that a special acquired predisposition is necessary. So far as we are aware there is no record of any case in which the administration of quinine to healthy persons has produced hæmoglobinuria. The ordinary effects of this drug upon the organism are well known and easily recognised; cinchonism or true quinine intoxication is a familiar phenomenon among those who have to make frequent use of the remedy; and its occurrence almost always bears some direct relation to the amount absorbed; hæmoglobinuria is not a symptom of this condition, and has never to our knowledge been recorded as occurring in the case of persons in whom the accidental administration of an overdose has produced fatal results.

It is true that more or less concentrated solutions of quinine will *in vitro* produce hæmolysis of human blood corpuscles suspended in salt solution; but as will be seen later, when we give our own observations, it does not exercise this effect upon red cells suspended in human blood serum; and there is no evidence to show that quinine exerts a directly destructive effect upon normal human erythrocytes within the body.

Obviously the action of quinine in these cases must be clearly distinguished from that of such a substance as chlorate of potash, which, innocuous in small doses, in larger quantities produces markedly toxic symptoms, prominent among which is red cell destruction and hæmoglobinuria (methæmoglobinuria). Administered in poisonous doses, the action of this substance upon a person can be foretold with certainty, quite apart from any consideration of such questions as special predisposition, place of residence, country, or any other circumstance.

But quinine never produces hæmoglobinuria except in the case of persons who are the subject of predisposition, acquired only after residence under special circumstances, in certain places; and even in such persons the occurrence of hæmoglobinuria can never be prognosticated with any certainty, save in a fractional percentage of cases. That cases do exist, in which this acquired idiosyncrasy is so marked that even the most minute dose of quinine is followed within a few hours by the passage of hæmoglobinuria urine has been well established; and it is the recognition of this fact which has led to the attempt to distinguish a special form of quinine hæmoglobinuria in contradistinction to the class of case in which the relation of quinine administration to the onset of this symptom is not so evident, or in which quinine can be definitely excluded.

Many cases of Black-water Fever occurring without the administration of quinine have now been recorded by A. Plehn (4) (twenty-two); Cardamatis (5) (thirty-two); Theophanidis (5) (ten), Korylos (5) (three), Shropshire (8) (fifteen per cent. of apparently one hundred and seventy-seven cases); and many other observers.

III.—That Black-water Fever is a distinct disease due to some Specific Organism.

As a result of the kindling of interest in Tropical Parasitology, which followed upon Manson's and Ross's discovery of the mosquito cycle of the malarial parasite, protozoal infections in animals (such as the piroplasmoses) have been brought prominently before the minds of those engaged in tropical research. The importance of not overlooking new human parasites was still further accentuated by the discovery of the parasite of Kala-azar, and the danger of confusing diseases of somewhat like symptoms rendered very clear by the fact that up to this time infection by the Leishman Donovan Body had been everywhere classed as malaria.

Even in 1898 the apparent resemblance shown by Black-water Fever to the hæmoglobinuria of animals associated with piroplasma infections naturally presented itself to anyone studying the disease. No one has succeeded, however, in spite of much attention, in finding in the blood or organs or 'Black-water Fever cases a special parasite of this nature. Such a view of the origin of the disease is therefore at present not supported by any facts; and it is even made somewhat improbable by a study of all that is known of the condition, notably the amount of positive evidence pointing to a malarial origin, and the difficulty of fitting in a special parasite view with what we know of the action of quinine in this disease. The demonstration of the presence or absence of a special parasite is, however, of such fundamental importance, that until absolutely disproved the possibility of its presence must be given every consideration.

IV.—That Black-water Fever is the result of an induced condition brought about by Repeated Malarial Infection lasting over a certain Time.

Most modern observers have accepted this view, first brought forward by Stephens and Christophers (9) and later strongly supported by Panse (10).

These observers, while agreeing so far with Koch that the morbid process concerned cannot be considered as one bearing any true resemblance to a malarial attack, formed the conclusion that it was exposure to severe and long continued malarial infection which brings about the condition of unstable equilibrium in the blood necessary before quinine, or any other determining factor, can bring about an attack of the disease.

Since then much confirmatory evidence has been amassed showing that Black-water Fever is clinically most closely associated with malarial infection. But this view of the origin of the disease is not universally accepted, and certain objections have been raised, more particularly by those who maintain that the disease is a specific one with no real relation to malaria. This being so it will be necessary to pass in brief review the facts that have been ascertained in regard to the relation of Black-water Fever to malaria, and to consider in detail the objections that have been raised against the theory that it is of malarial origin.

Geographical Distribution.

The geographical distribution of Black-water Fever we now know to be very wide. In Europe it is recorded from Italy, Sicily, Sardinia, Greece, and Spain, from Merv in Russia along the banks of the Danube, in the Caucasus and from Turkey. In Italy as Stephens (11) points out it occurs more especially in South Calabria, where the mortality from malaria, according to Celli, reaches 9 to 9·9 per 10,000, a rate double that in the Roman Campagna and more than ten times that in Northern Italy.

In Asia the disease has been met with in Cochin China, Tonkin, and Siam, and it occurs also in India, Burmah, the Straits Settlements, Java, Sumatra, New Guinea, and the New Hebrides; it has also been described as occurring among certain communities in the more malarious parts of Palestine.

Throughout Tropical Africa it is the chief cause of mortality among Europeans; and it is found also in Madagascar, Bourbon, Mauritius, Reunion, and the Comorro Islands.

In the West Indies it was known to the early French observers; and in Nicaragua, Costa Rica, French, British and Dutch Guiana, Brazil, Central

America, and Cuba it has been recognised for a considerable time; while American physicians from some of the Southern States were among the first observers to describe the disease, which has a wide distribution throughout the more malarious portions of that country.

That this distribution of Black-water Fever agrees in the main with that of the greatest intensity of malaria is evident; but it has been objected:

(1) That in the case of many of these countries the disease has only recently been introduced.

(2) That the coincidence with malaria is not exact; and more specially that there are countries notoriously malarious in which Black-water Fever is very rare, or does not exist.

The converse of this latter suggestion, namely, that Black-water Fever occurs in comparatively non-malarious districts has not so far as we are aware been advanced.

(1) The belief that Black-water Fever had a peculiar and restricted distribution has been emphasised in most text-books until quite recent years. To explain the constantly increasing area from which the disease is now being recorded some writers have advanced the view that in many of these countries, as, for example, India, the disease has only recently been introduced. This does not seem to us a conclusion warranted by the facts.

In making an examination of the literature of Black-water Fever one cannot help remarking that the recognition of its geographical distribution has followed periods in which the different nations became acquainted with the disease in their various colonial possessions.

The first may be called the French Period, and dates from the time when Lebeau (12), Daullé (13), and Le Roy de Mericourt (14) first described the disease "Fievre Bilious Hæmaturique" as occurring in Madagascar and some of the adjacent islands. Almost immediately other French physicians reported finding the same type of fever in Senegambia, Senegal, Gaboon, Guiana, the Antilles, and other parts of the West Indies.

Until 1858 nothing was heard of hæmoglobinuric fever outside French possessions. But in that year Verratas (15) a Greek, described what he termed "Quinine Hæmoglobinuria"; and two years later a similar communication was made by another Greek, Papabasilos (16).

The American period commences from about 1860. The disease was first described by Doctor Cummings (17) of Louisiana in 1859, and it was shortly afterward srecognised in Alabama and Texas (1866). From this time onward it was reported with increasing frequency from many parts of the Southern States of North America.

The Italian period may be said to date from 1874 when Tomaselli (6) pub-

lished his classic monograph upon Quinine Intoxication, describing cases of hæmoglobinuria seen by him in Sicily. Almost immediately, interest being aroused, the disease was shown to occur also in Sardinia and Southern Italy.

About this time too the Dutch physicians (Jacobs) (18) in Java, Guinea and New Guiana discovered the presence of the affection in those colonies.

From 1890 commences the German period, with Schellong's (19) well known work in Kaiser Wilhelm's Land; and from this time, co-incident possibly with the stimulation of German colonial activity, we find numbers of investigators belonging to that nation recording observations upon the disease from the Kameroons, German East Africa, and New Guinea. Prominent among the names of these workers are those of Grawitz and Kohlstock (20), Steudel (21), the two Plehns (3 and 4), Ziemann (22), and Koch (7).

Up to comparatively recently little or nothing of the disease had been recorded from British dependencies although French observers had already reported it from various parts of Africa, Asia, and America; and though Crosse (22*a*) and Manson (22*b*) had called attention to the disease in 1892, and from time to time a few isolated papers upon the subject had appeared in English medical journals, no adequate account of the condition had yet found a place in text-books available to the majority of British physicians practising abroad.

But in 1898 as an immediate result of Manson's and Ross's great discovery a sudden and extraordinary awakening of interest in Tropical Disease was aroused throughout the British Empire, and within the half dozen or so succeeding years we find Black-water Fever recorded by British observers from British Central Africa (Moffat) (23), Stephens and Christophers (9), Daniels (24), Hearsey (25), India (Powell) (26), Seal (27), British Honduras (Brown) (28), Rhodesia, Uganda (Moffat) (29), Nigeria (Hanley) (30), British East Africa (Daniels), the Soudan (Ensor) (38), China (Wenyon) (31) British Malaya (Wright) (34), Burmah (Finke) (35), Syria (Cropper) (36), Masterman (37), and elsewhere. It is obvious that a recent introduction of the disease into these countries is most unlikely.

(2) In regard to the objection that the distribution of Black-water Fever is more restricted than that of malaria, Stephens has pointed out that, in many countries where malaria is known to occur, the inhabitants are not exposed to the disease in an intense form; and that if we consider the distribution of intense malaria we shall find that it coincides very closely with that of Black-water Fever.

It has been stated that the distribution of Black-water Fever is not that of any of the known varieties of the malarial parasite; but it is difficult to see how such a statement can be upheld for all we can say at present in regard to the distribution of the different varieties of malaria is that, as one approaches the tropics,

one finds infection with the malignant tertian form more and more frequent, a statement which applies also to the frequency of Black-water Fever; and that Black-water Fever can arise from only one variety of the malarial parasite we have at present no proof.

It is then only with the intensity of malaria that we are concerned, and the only portion of the objection that seems to merit consideration is the statement that there are certain countries which are intensely malarious in which Black-water Fever either does not occur or is very infrequent. Though it is repeatedly averred that this is the case, the basis of fact for such a statement is extremely slight.

It has been stated that Black-water Fever is rare in Algeria; but the brothers Sergent (39) have within recent years recorded 31 cases with 22 deaths, and Coste (40) has reported another 25 cases, proving that the condition is far more frequent than has previously been supposed.

Black-water Fever is also stated not to occur in Egypt, a very malarious country; but it is significant that it is common in parts of Palestine; and it is being reported from the Soudan north of Fashoda.

The greatest stress of all is laid on India. It has been stated that, whilst people in India are much subjected to malaria and are accustomed to take quinine freely, there is no Black-water Fever; and it is the same view which has caused this country to be cited so frequently in the present connection.

Manson (42) emphasizing the objection to the malarial origin of the disease says, speaking of Black-water Fever: "It is exceedingly common among the few Europeans who live in Tropical Africa, it is practically unknown amongst the many thousands of Englishmen who live in the fever haunts of India."

These views would no doubt be readily echoed by numbers of medical men in India, who affirm, and we believe often quite rightly, that they have never encountered the disease.

At first sight therefore it might appear that there were grounds for the assertion that in India while severe malaria is common Black-water Fever is generally speaking, rare or absent, and should this view prove on enquiry to be correct, it is obvious that it would form a serious objection to the theory based upon the suggested intimate relations between these diseases. Before giving weight to this objection it is necessary to consider the history of Black-water Fever in India and to note the circumstances in which it has appeared.

Maclean (43) in his lectures upon the Diseases of Tropical Climates (1886) states, with reference to the urine in certain cases of remittent fever, that "In some cases recorded by me there was profuse secretion of bloody urine, which lasted until convalescence set in." But Powell (26) was the first actually to

recognise the disease, when he recorded the occurrence of a number of cases in Cachar.

A little later other cases were reported from Sylhet, the Darjeeling Terai, Cachar, and the Duars. Stephens and Christophers, who described four cases from the Duars, also note in their report to the Royal Society that cases had been met with in other parts of India, including Hazaribagh, Meerut, Roorkee, and Secunderabad, where the disease is common among the Europeans in the Jeypore Hills. They also mention that cases of the disease have been seen from time to time in the hospitals of Calcutta and Madras, though these may have originated in other places.

Since then [illegible] (32) has recorded three cases from Canara in the Bombay Presidency, and DeCruz (33) a further number of cases among both Europeans and natives in the Jeypore district.

Nor are these the only places in which Black-water Fever may occur in India.

In our list of cases given in the present memoir we are able to record one in a European from Purulia in Chota Nagpur; and Colonel C. J. Bamber, I.M.S., has drawn our attention to the death of a European officer from Black-water Fever in the Lower Godavery district, a reputedly malarious part of Southern India. Doctor Gopal Chatterjee has also told us of two cases in Bengalis living near Calcutta, where he states there are some very malarious villages. The family in which both cases occurred had for some time been suffering from malaria, and he had on several occasions found them to have malarial parasites in their blood. Doctor Bramacheri has given us particulars of a case arising in a native of the Burdwan district.

These represent, we feel sure, only a few of the districts in which the disease may be occasionally met with. Many other likely areas probably have no European residents; but in others where Europeans and educated natives reside we suspect that careful enquiry would show the disease to exist.

A common belief, that a disease having such a striking symptomatology as Black-water Fever can scarcely be overlooked, is not borne out by experience; it is only too frequently entirely unrecognised or confused with other diseases, when it occurs in districts in which it has not previously been diagnosed. As a rule where Black-water Fever occurs, men are for the most part living remote from civilization, and the disease being a sudden one and soon over a case except under special circumstances is often not seen by any medical man. Again in all such communities "fever" is only too familiar, and, even when a man is seriously ill, he often thinks it unnecessary to call in medical advice though this may be available.

It has been supposed that the patient himself must be struck by the extraordinary change in his urine; and no doubt he is if he knows that the disease from which he is suffering is Black-water Fever; but in a district where it is not believed to exist, and among a community who have only heard vaguely of the dread disease, a man may overlook the condition of his urine and not suspect that he is suffering from Black-water Fever.

All these points are well exemplified in the case I have referred to from Purulia. It was only because he was travelling to Calcutta at the time and was taken to the General Hospital that this man came under observation; also in spite of passing though a typical attack of the disease he did not know that he had Black-water Fever, and had he been in his own district the attack would certainly have gone unrecorded, for he and one other European were living alone in a remote part, many miles from the nearest civil station.

Black-water Fever therefore does occur in India; but it is evidently confined to certain localized tracts, and there can be no doubt, as has been maintained, that it is rare in the practice of the average Indian physician. *In this fact lies one of the most powerful arguments for the view of the malarial origin of Black-water Fever, yet based on the grounds of geographical distribution.*

The areas we have especially mentioned as centres of Black-water Fever are all tracts at once differentiated from the usual conditions in India by the intensity of their malaria. Assam, the Duars, and the Darjeeling Terai we shall consider in detail later. It is only necessary in the present connection to say that they are notoriously malarious districts.

The Jeypore District is a hilly intensely malarious tract, where the European and native residents alike suffer severely from malaria.

Canara, on the west coast of India, is also a hilly district with a heavy rainfall, and it is interesting to note that before the occurrence of Black-water Fever was actually recorded by Christie, Stephens and Christophers, judging from physical and other features, had already indicated this district as one likely to yield cases of Black-water Fever.

Chota Nagpur is an extensive tract of hill country largely occupied by aboriginal tribes, mentioned by Hirsch as one of the most malarial parts of India.

Later on in this memoir we shall be able to picture what residence in such districts entails to the European and even to the native of India.

But these conditions are not general throughout India in spite of an impression to the contrary among those who are unfamiliar with life in this country. Malaria exists as a rule, it is true, but in a degree in no way comparable to its intensity in the Black-water Fever districts. Europeans also, for reasons into

which we need not enter here are usually but little exposed to such infection as exists.

Thus one of us, [illegible] a system of experience, found it quite unnecessary to adopt special precautions against malaria while living in the outskirts of Madras; nor did those living in the [illegible] neighbourhood suffer, though a certain amount of infection, by no means great, was to be found in the bazaars. This condition is experienced in Calcutta, Rangoon and most Indian stations and is met with even in rural districts, for one [illegible] passed two years in a portion of Assam (Cachar) without contracting fever, although no special care was taken to avoid it; but on moving to [illegible] Black-water Fever district, he suffered severely until from necessity [illegible] the practice of taking quinine daily. Again in many [illegible] tracts of India malaria may be very rife, but only at certain seasons. Thus at Miani Mir which is one of the most notoriously malarious stations of the Punjab, malaria is only really prevalent for two or three months in the autumn; and this remark applies more or less to the whole North-West, where rainfall is scanty and there is a long period of drought associated with a cold season.

Briefly stated then we may assert that the great majority of Europeans in India are not exposed to Black-water Fever conditions, assuming that these are rife in an intensely malarious district. An objection has been raised in regard to the British soldier, who is frequently exposed to malarious conditions and often suffers severely from the effects of residence in a malarious station, but apparently does not suffer from Black-water Fever. The British soldier, however, is under continual medical inspection and is almost certain if he suffers much from malaria to be invalided or drafted to some healthier place long before the necessary condition of predisposition has been reached.

It is evident, therefore, after a full consideration of the facts, that the arguments raised against the theory as to the malarial origin of Black-water Fever based upon the geographical distribution of that disease are no longer tenable in so far as they relate to India; for an examination of the real facts shows that where in India there is intense malaria Black-water Fever is also found.

Association of Black-water Fever with Malaria.

That it is among communities living under constant liability to attacks of malaria that Black-water Fever is found to exist is very generally admitted; but it has been urged that this association of the two diseases is only accidental, due merely to the fact that the conditions favourable for the occurrence and propagation of malaria are also suitable for the existence of the special parasite, which has been suggested as the cause of Black-water Fever. It is a very important matter then to decide whether the association of the two diseases is mere

concurrence or an actual relationship. Stephens, supporting the view of the malarial origin of Black-water Fever, brings forwa[illegible] evidence for such real relationship based on the results of the examin[illegible] he blood and tissues in Black-water Fever. He tabulates the results ob[illegible] competent observers in ninety-five cases, and shows that in 95 per cent. [illegible] observed the day before the attack malarial parasites were present. [illegible] points out that the failure to find parasites in cases of Black-water [illegible] n at a later stage in no way invalidates the fact that an initial malarial [illegible] on is almost always present. Both he and Christophers had previous[illegible] attention to the subsidiary signs of malarial infection almost invariab[illegible]nt in Black-water Fever even when parasites were absent. Thus provi[illegible] Fever were [illegible] be seen fairly early although no parasites may be detected careful sea[illegible] will frequently [illegible] strate the presence of pigmented leucocytes—undeniabl[illegible] evidence of very recent infection: moreover a differential count of the leucocytes will usually show the large mononuclear increase commonly met with in malarial bloods. With few exceptions also examination of the tissues of fatal cases has nearly always shown the presence of parasites or pigmented leucocytes, the latter being so distributed as to point to very recent infection.

In accord with such observations are others of a clinical nature, and it is significant that in Italy where the study of malaria has been carried on with great thoroughness and minuteness, *hæmoglobinuria of malarial origin has been fully recognised.* Marchiafava and Bignami, discussing cases of hæmoglobinuria, draw attention to the fact that these conditions should be included under post-malarial phenomena, that is those which occur not only after the cessation of the fever, but even when parasites have entirely disappeared.

In regard to the disease in the tropics one is forced also to the conclusion that if Black-water Fever is not caused by malaria it is closely bound up with it, so much so that, whenever a case is seen under conditions suitable for accurate observation, it is almost invariably found that the attack has been associated with an immediately preceding attack of malaria. Panse's cases are exceedingly convincing in this respect; studying patients in Europe, who suffered from relapses after return from Black-water Fever districts, he frequently saw the onset of Black-water Fever in the course of the malarial attack for which the patient had been admitted, and with the progress of the case noted the more or less rapid disappearance of the malarial parasites.

In addition to these there are other facts of a more general nature which must be given due consideration.

A most noticeable feature of Black-water Fever is that it occurs only in those who have been resident for some considerable time under Black-water Fever conditions; fresh arrivals are never attacked. Berenger Feraud (44), in

185 cases, has seen only one under three months' resident, the remaining 184 occurring as follows:—

Under one year	10
Second year	42
Third year	79
Fourth year	37
Fifth year	9
At some later date	8

This feature of the disease is of the greatest importance from an ætiological point of view. It has been argued that if Black-water Fever were a specific disease it would not attack persons just at this period of their residence. It is suggested on the other hand that this peculiar incidence is explicable as due merely to the accumulating chances of infection as time goes on. Such a view, however, seems improbable in the face of the very remarkable uniformity in the length of residence necessary before infection takes place. Later on we shall see that in India at any rate this peculiar incidence cannot be explained as a result of concomitant circumstances.

Quite a different state of things is found in malaria in which disease it is customary for new-comers to a badly malarious country to contract infection very shortly after their arrival; and the same holds good in regard to susceptible animals brought into countries where piroplasmosis is endemic.

Again there are no grounds for the belief that any single attack of malaria, however severe, can give rise to hæmoglobinuria except in a subject who has been for some time resident under Black-water Fever conditions; Black-water Fever cannot therefore be a complication of a mere attack of malaria.

Another well recognised fact in regard to Black-water Fever is that it attacks only certain susceptible races whilst the indigenous inhabitants of the countries where the disease is prevalent apparently do not suffer. Thus in Africa besides Europeans, Syrian hawkers, Indians, and even Negroes who have not been born and bred under these conditions are liable to attacks. This is in exact accordance with what we know of malaria in such countries as Tropical Africa, where the adult among the indigenous population possesses an immunity acquired by repeated infection in childhood. The non-occurrence of Black-water Fever among these people suggests either an intimate relation of such immunity to malaria or the assumption of an exact parallel in the course of infection by some other parasite.

That a man may develop Black-water Fever, even for the first time, after his return to Europe, is a fact generally recognised; and many cases occur too long after leaving a district to make it likely that the disease has been merely incubating. Of all diseases known to us malaria exemplifies this peculiarity

most, and that Black-water Fever also shows it is a point not to be lightly dismissed. Panse's observations previously quot[illegible] that such attacks ar invariably preceded by an active malarial infection[illegible] is at once of course possib to explain attacks under such circumstances by st[illegible] hæmog[illegible] that the malaria ha lowered the patient's resistance to the latent caus[illegible] produce [illegible]-wate[illegible] Fever, which then asserts itself: or that the latter has stimulate[illegible] the [illegible] malarial parasite into activity. In either case we must allow that Bl[illegible]t-hæmogl[illegible] Fever, like malaria can remain dormant in the system.

[illegible]subsequ[illegible]y

[illegible] of b.oo

normal

THE PLACE OF QUININE IN THE MALARIA HYP[illegible]OF [illegible]R[illegible]
OF BLACK-WATER FEVER

[illegible]e to unde[illegible]n.

[illegible]e r[illegible]ard

There is no incompatibility between the view wh[illegible]h asser[illegible]tha[illegible] quinine administration may produce Black-water Fever and the malari[illegible]pothesis, we admit that the drug is only a determining cause, act[illegible]ng in co[illegible]tion with a condition induced by previous intense malaria. The malaria hyp[illegible]esis certainly explains more readily than any other the undoubted action frequently exerted by quinine in the precipitation of an attack of hæmoglobinuria.

Thus far we have summarized the recorded facts relating to the remote[illegible] causes which act in the production of Black-water Fever.

As to the mechanism and the exact process concerned in the terrific destruction of red cells we have no certain knowledge; but as may be expected this has been the subject of much speculation and conjecture.

The earlier writers held that Black-water Fever occurr[illegible] only when the red cell destruction, usual to a malarial attack, exceeded a c[illegible]tain point. It was thought that the liver, being then unable to cope with the [illegible]xcessive amount of hæmoglobin liberated allowed a portion of this to pass uncha[illegible]ged into the urine. Modifications of this view are that Black-water Fever is the [illegible]sult of a peculiar virulence of the malarial parasite brought about by certain conditions, such, for example, as their transmission through a particular species of anopheles (Daniels); or that it is due to an excess of toxines liberated by the parasite possibly under stimulation of quinine (Grocco) (45).

The view that asserts Black-water Fever to be a specific disease and draws an analogy between it and the piroplasmoses hypothecates that instead of the red cells being destroyed by the malarial parasites or their toxines, the effect is brought about by a special parasite.

The action of quinine in liberating hæmoglobin from the red blood corpuscles has been urged, and as Stephens and Christophers pointed out solutions above a certain strength possess the power to hæmolise red cells. Marchoux (46) moreover asserts that quinine is not excreted during the actual period of Black-

water Fever; but that its presence may be demonstrated in the urine as that condition subsides. Be... des (63) belives that quinine has no action on intact d cells; but that on its coing up of these cells, it may act on the liberated æmoglobin converting nd the methæmoglobin, which is at once excreted by the tidneys as a foreign omers ha He suggests that the hæmoglobinæmia resulting from an ordinary at does no malaria does not usually produce hæmoglobinuria, ormal hæmoglobin bei s spily absorbed, but that with the presence of quinine the blood the reacti bjectiored to takes place, and met-hæmoglobinuria results.

A theory adva ec mere layer (47) in 1898, and subsequently adopted by ully others as a poot planation of the mechanism of blood destruction, a ed that b o became less resistant than normal to changes in th ot an sion o plasma, and thus became liable to undergo solution. Stephe anom hristophe , investigating the condition of the red cells in regard their be r in diffe ent strengths of salt solution (isotonic point), found that during tack of Black-water Fever they might show an even increased resistance to tion; though the examination of the red cells of persons living under Black-wa on Fever conditions frequently showed them to possess less than the normal resistance. They conclude, therefore, that the apparent raised resistance observed in Black-water Fever might be due to the destruction of the weaker cells in the course of the disease, with the result that only the most resistent cells remain after an attack. Certain French workers have suggested, as an explanation of the phenomena observed in Black-water Fever, the occurrence of what they termed "demineralisation of the plasma," a condition which they assume a reduction in the amount of salts normally found in solution in the plasma, the resulting alterations in osmotic conditions giving rise to destruction of the red corpuscles. More recently McCay (48) has brought forward an interesting hypothesis based on the observation that after the administration of even a single dose of quinine sulphate, he finds a reduction in the salt contents (hæmosozic value) of the plasma. This he ascribes to the action of the acid radical and not to the alkaloid, as his observations caused him to believe that the administration of quinine hydrochloride does not produce the same effect. In the main his theory regarding Black-water Fever is in agreement with that of the French observers previously alluded to, the process of destruction being supposed to be the result of the "demineralisation of the plasma." But up to the present no observer has given sufficient data to enable an opinion to be formed as to the part played, if any, by osmotic variations in the production of Black-water Fever.

Certain other theories have from time to time been put forward, the mention of which serves to indicate the general confusion and uncertainty that has for long existed regarding the process concerned in the blood destruction of

Black-water Fever. Thus Pellerin (49) advance the view that the kidney was involved, the hæmoglobin observed in the urine b[illegible]ived from hæmorrhages into that organ or from the breaking down of infa[illegible]'lehn has also cite as a possible explanation a damaged condition E PRESE[illegible]idneyS[illegible]n some wa altering their relation to the blood which, in the ca[illegible]al malarial subjects might suffer injury and be deprived of its hæmo[illegible]in parts its passage through them. Yersin (49*a*) announcing the discovery of [illegible]moglob[illegible]us [illegible]the urine suggest its toxic effects upon the blood as a likely cause; hæmoglob[illegible]let (49*b*), influence perhaps by the fact that the filtrate from cultures o[illegible]rtance a[illegible]3. *legatherium* m[illegible] excite hæmoglobinuria when injected into guinea-pi[illegible]t it is bec[illegible]nentioned oss[illegible] infection by that organism as a question to be consid[illegible]ns in t[illegible]al an[illegible]es.

But of the real mechanism of Black-water Fe[illegible]d an enor[illegible]us lit[illegible]ature, though certain observers, prominent among whom are [illegible]sis Italians atm[illegible]ve [illegible]of the formulated the view that Black-water Fever is due to [illegible]he actio[illegible]f a specific hæmolysin, and one of them Casagrandi (50) states t[illegible]at he h[illegible]r les[illegible]cceeded in demonstrating in malarial hæmoglobinuria such a hæ[illegible]olysin someti[illegible]ed by the presence of an anti-hæmolysin. The study of the c[illegible]mplex and r[illegible]omena into which these researches lead us is better discussed in re[illegible]tio[illegible] to our own later investigation.

CHAPTER II.

NATURE OF THE DISEASE. SCOPE OF THE PRESENT ENQUIRY.

Black-water Fever is a disease localised to certain parts of the world; it is characterised by hæmoglobinuria. Apart from it hæmoglobinuria in man is rare being seen only in occasional cases of paroxysmal hæmoglobinuria or as a rare complication in certain [illegible] diseases. On its importance as a bar to Colonial expansion we need not comment, beyond saying that it is becoming more and more recognised as [illegible] of death among Europeans in tropical countries. [illegible] striking symptomatology, and an enormous literature [illegible] on the clinical features, diagnosis, and treatment of the [illegible] appe[illegible] **Definition.** **Importance.** **Clinical features.**

[illegible] attacks as a rule men who have been more or less affected by the [illegible] to present in tropical malarious countries, but sometimes the disease strikes [illegible] an who to his companions seems strong and robust.

There occur [illegible] most cases what have been called prodromata. It is [illegible] by Nothnagel [illegible] these prodromata which resemble malaria differ in presenting a more marked cold stage. Our own experience is that the prodromal fever can never be differentiated from an ordinary malarial attack, which patients themselves almost always consider it. Whether it is so in reality we must leave for further discussion. In some cases an intermission of the prodromal fever is followed by the Black-water which comes on as though it were the fever returning, while in others no definite prodrome has been recognised. **Prodromata.**

Almost invariably a rigor followed shortly afterwards by the passage of hæmoglobinous urine marks the onset of the disease. In the history of some cases it is stated that the abnormal urine was the first symptom, and that the rigor followed, but this point we cannot ascertain with certainty. The urine first passed may be light red in colour or quite dark. In an ordinary case it is lighter at first but soon becomes of a deep mahogany red colour or even nearly black, though even then it appears claret coloured by transmitted light. With slight variations these characters of the urine continue until hæmoglobinuria ceases or suppression supervenes. At the onset the temperature generally rises to 104° F. or even 105° F. and remains high during the greater part of the paroxysm. But sometimes, especially in mild cases, the disease runs an almost afebrile course. Throughout the attack there is often extreme thirst, and usually great nausea; and the bilious vomiting with the jaundice has served to give the disease the name of "Bilious Hæmoglobinuric Fever." **Onset.** **General Symptoms.** **Temperature.**

In severe cases the patient is restless, complaining of want of air and with all the appearance of an acute hæmorrhage case. A symptom which may be

persistent and distressing is pain over the epigastrium, and tenderness may be present over the spleen, which is nearly always enlarged before the liver.

Jaundice. At the end of the first twenty-four hours or so, sometimes earlier, a slight icteric tinge begins to show itself and rapidly increases in intensity, though rarely equalling in degree the profound yellow colouration sometimes seen in such other forms of destructive jaundice.

Urine. The urine which at the commencement of the attack may be bright red, resembling laked and diluted blood, shews on spectroscopic examination the absorption bands of oxy-hæmoglobin. Later it becomes darker and more brownish in colour, appearing nearly black when held between the eye and the light in bulk. After remaining for some time more or less uniform in tint, the visibility of the spectrum suddenly becomes less intensely coloured and finally quite [illegible]. It is common to see in the course of an attack, especially [illegible] are one or more periods of exacerbation shown by rigors, Fever, and [illegible] colour of the urine. Not infrequently the urine may clear [illegible] globinuria to return with a new accession of the symptoms, or to cut short its after the hæmoglobinuria there is urobilinuria which [illegible]. Albuminuria which during the hæmoglobinuria is often sufficient to cause the urine to become nearly solid on boiling continues in a [illegible] degree for many days after.

Blood. The blood at first not markedly anæmic eventually becomes in a severe case pale and watery, the red cells being sometimes reduced to less than a million per c. m., whilst the hæmoglobin value is correspondingly low. Very early in the attack a few malarial parasites are usually present, but later they are not found even after the most prolonged search.

Recovery. If recovery takes place convalescence is rapid, and the patient often has a spell of better health than before the attack of Black-water Fever, but if he be exposed to the same conditions as before a second or third attack is only too liable to follow. Each time the chances of a fatal termination are very great.

Death. When death takes place it usually follows suppression of urine, the result of a profound effect upon the kidney epithelium. At other times the enormous blood destruction itself leads to a rapidly fatal end. Even if the patient pass safely through the actual hæmoglobinuria he may die many days later from obscure causes.

Morbid Anatomy. Post-mortem the spleen is greatly enlarged, congested, and dark in colour. The liver also is enlarged, and congested, the kidneys enlarged and dark purple in colour. Almost characteristic of the viscera in Black-water Fever is pigment of malaria, a disease which if it be not the cause of Black-water Fever is everywhere associated with it.

This then is the disease. It is necessary for us after we have summarized what has been ascertained and surmised by other observers as to its nature to give our own contribution to its study.

Our researches upon Bla[illegible]k-water Fever have been carried out in the Duars, a district which has [illegible] notoriety in India as one where Black-water Fever is prevalent. Our [illegible] as to ascertain the conditions under which Black-water Fever occurred [illegible] collect such general information regarding seasonal and local incidence, the [illegible] race, length of residence, and such other conditions as seemed likely to be of [illegible] importance in determining the cause and nature of the disease. Having [illegible] d as far as possible the general causes bringing about Black-wa[illegible] there still remained for consideration the immediate mechanism conce[illegible] disease process, and the question as to how that mechanism was [illegible] ction. Lastly, there is the possibility of suggesting a [illegible] phylaxis for the disease.

[illegible] In these [illegible] erefore were before us :—

the young [illegible] en, appe[illegible] he causes bringing about Black-water Fever ?
[illegible] on suc[illegible] actual mechanism of the blood destruction ?
by the [illegible] to pr[illegible] can be taken to prevent the disease or to cut short its disease strik[illegible] on by [illegible] nce it has declared itself?

The[illegible] mon o[illegible] to chiefly engaged the attention of observers, and the Duars ; no[illegible] first has [illegible] at the present time has been briefly outlined.

[illegible] ate of our knowle[illegible] to explain the cause of the condition which seem at all

The only attemp[illegible] time are :—

applicable at the pre[illegible] that Black-water Fever is a specific disease.

(*a*) The the[illegible] nduced by repeated attacks of malaria, quinine occupy-

(*b*) That it [illegible] minor position as a precipitating cause.

ing [illegible] nd it is necessary to consider the possibility of its being

On the other [illegible] ndition not yet indicated.

due to some other [illegible] ide upon the probability or improbability of each of these

We have to [illegible] g forward if possible proof of their truth or the reverse.

suggestions, bring[illegible] stigating these remote or general causes we wish to approach

But while in[illegible] ck-water Fever from a new standpoint, bringing as many the subject of [illegible] ssible to bear upon the mechanism of blood destruction in the observations as [illegible] n these lines does a true solution of the difficulty surrounding disease. Only [illegible] ack-water Fever seem possible ; it is also only with a knowledge the ætiology of B[illegible] hope to approach the rational treatment of the condition. of this that we ca[illegible]

PART II.

Blac[illegible] Fever malarial in origin.

CHAPTER I.

[illegible]VATER FEVER CONDITIONS.

A glance at a [illegible] of India shows at once two areas where the rainfall [illegible] that [illegible] ne rest of India. One is a narrow strip along the West[illegible] Coast. The other is situated along the base of the E[illegible] Garo and Kassia Hills, where these ranges intercept the youre[illegible]en, appe[illegible]n currents from the Bay of Bengal; within its limits [illegible]opeans [illegible] on suc[illegible]tricts of the Darjeeling Terai, the more important by the ill[illegible]e to pr[illegible]s and the still more important and extensive Province disease strik[illegible]on by [illegible]

Ther[illegible] with which we are entirely concerned, since it will be [illegible] common o[illegible] area from which cases of Black-water Fever have Duars; nor [illegible] correspond[illegible] 1898 Powell (26) records the occurrence of Black-th[illegible]een frequently reporte[illegible]g eleven cases which had come under his observation in North Cach[illegible]ortly afterwards Seal (27) gave particulars of a case seen by him in S[illegible]other district of Assam, and six other cases in the Darjeeling Terai. In[illegible] Rev. G. M. Crozier, M.D. (51), described two other cases from Ca[illegible] a little later Stephens and Christophers noted in their report to the R[illegible]ciety that the disease was frequent in the Duars, and also referred to [illegible]received by them from an Assam planter mentioning that it had been [illegible]ed for a number of years in Nowgong.

Since then the [illegible]s gained a notoriety far above Assam as a Black-water Fever countr[illegible]ases have come under the personal observation of one of us in the Da[illegible]rict (Assam), while particulars of others have been sent to us from va[illegible] of the province.

To what condi[illegible] the district under discussion owe the occurrence of Black-water Fever [illegible] is the Duars especially the seat of the disease?

[illegible]NING UP OF THE SOIL.

The belief that se[illegible]ria is often the result of opening up the soil has long been held. To ex[illegible] it has been customary to note that pools are generally formed suitab[illegible]pheles to breed in, and that malaria is the result; yet in reality thi[illegible]an inadequate conception of the true state of

affairs. We have been fortunate enough to see in several parts of the Tropics operations involving " Opening up of the soil." Situated erations always involve certain conditions. The most important is the for rice of enormous camps coolie labourers and their families, drawn fromcome per widen larger or higher district, or even from distant countries. In such up around ar more mixed than mere extra facilities for breeding of anopheleome spertain con wider mixture of races and classes involving admixture o fact tipti ies number of ed ; the depressing effects of hardship, especially rden wo g throws greater turn by becoming malarial disseminators take thel facto intending to im- crowding of the population into communities larger he incidence of malaria conditions and not large enough to derive the benefit to some extent among and many other factors we need not specify. Suffice but little ; and those ditions play into the hand of malaria more than any otatively little from the in these huge labour camps we find malaria in its inteture that Assam as a form.

Naturally the Europeans and others employed in intains opening ition general trouble, since in their ignorance they dwelle vic exhalations or eve. midst of the natives. The belief that malaria comes from ure this too helps in the soil is therefore based upon fact ; but it is not the miasm mund up in the general the extra facilities for the breeding of anopheles, thoug camps in the Tropics. general vicious cycle, that is responsible. Its origin is b call it the factor " of conditions inseparable from the existence of great labourria

To emphasize the condition, which is a very real one, we m ed up and occupied Tropical Industrial Aggregation."

Much of the area we are discussing has only been op or virgin forest It within the last fifty years and vast tracts are still jungle hich it is the chief owes its recent importance mainly to the tea industry, of on the tea gardens, site ; and our interest in the district centres in the condition to speak. of which alone we have the knowledge necessary to enable tea-garden popu- character of such

When we come to discuss the incidence of malaria am gradual develop- lations, we find that there are several points relating to t oying large work- populations which require consideration. The introduction a supply of labour ment of the tea industry has resulted in the necessity of introduction of vast ing populations ; and the difficulties encountered in obtain from among the indigenous local population has led to e has gone on under numbers of immigrants from various parts of India. to some slight degree

Fortunately for Assam immigration in this p results in an immigrant a system known as " indentured labour " which regu more or less permanent. conditions of life and period of residence. This syste population it is true, but one with a tendency to become

The degree of permanenc[illegible], however, varies under different conditions on different gardens and is [illegible]bound up with the malarial intensity, the two factors forming a vicio[illegible]con Certain gardens are situated upon grants which are comparatively low-l[illegible]nd include land suitable for rice cultivation; as a result there is more i[illegible]mers ha[illegible] for the labourer to become permanently settled rather on the garden o[illegible]e villages which spring up around it. The fixed character of such popula[illegible]spread their tendency to become spread over a wider area than that allowed by [illegible]tionary garden lines, and the fact that the number of labourers being usually [illegible]more than is required for the garden work allows greater individual freedom a[illegible]s less arduous work, are all factors tending to improve the [illegible]of the population and lower the incidence of malaria among the[illegible]. In these [illegible]dens malaria, although present to some extent among the young children, appears to affect the adult population but little; and those Europeans [illegible]on such gardens usually suffer comparatively little from the disease. [illegible]e to preponderance of gardens of this nature that Assam as a whole is look[illegible]on by Europeans as fairly healthy.

But in [illegible]the yearly influx of new coolies maintains the condition of [illegible]temporary labo[illegible]amp year after year. Owing to the vicious cycles thus introduced the populat[illegible]is invariably of a more shifting nature, the tendency to gravitation of its meml[illegible]s to more healthy localities being more marked, the mortality higher, and [illegible]consequent introduction of new immigrants proportionately more frequent a[illegible]in greater number.

In Assam, gard[illegible]s under this category are almost invariably situated near the foot of the hills, [illegible]ch a situation in Assam being therefore generally associated with intense ma[illegible]ia.

If we ascertai[illegible]the districts from which Black-water Fever has been reported, we shall [illegible]nd that cases have almost all originated in gardens so situated.

Classified acc[illegible]ing to locality the cases of which we have the necessary particulars are :—

North Cachar	. 11 cases (Powell)	. . Gardens near the foot of the north Cachar Hills.
Budderpore	. 1 case	. Railway servant working on hill section.
Kumbhier	. 1 case	. Foot of north Cachar Hills.
Sylhet . .	. 3 cases	. One on a garden below Jaintia Hills; one on a garden near Lushai Hills; and one recurrence of a Duars case in a healthy district.

Bishnath . . .	1 case . . .	. On a garden near the foot of e en, the Himalayas.
Tezpur . . .	13 cases . . .	into the d gardens near the f t opulation ne Hima ayas.
North Lakhimpur .	3 cases . . .	widespre on gardens near the foo ere els the Hi alaya .
Lakhimpur . .	7 cases . . .	n place known to be i then tensely malarious.
Jorhat . . .	1 case . . .	he On a garden nea the Na Hills a
Nowgong . .	5 cases	On g dens under the Mikhir Hills.
Garo Hills . . .	3 cases	Elevation u hards .

But from the many gardens situated on the plains of Sibsaug. and Lakhimpur or other places comparatively healthy as regards malaria ter a single case has been recorded.

In the Duars we find a system known as "voluntai labour ease system which entails a great influx and efflux of labour far beyond th t in ter orst gardens on the "indentured labour" system. So marked is rting ch acter of the population under these conditions that we have t sun nique phenomen which resemble those the whole population of a district living under condition el s; and involved in the seen on any large engineering undertaking in the tropic i m the subject of our general scheme are the Europeans and Babus, who f e believes to be the present investigation, since it is they who, whatever ma n by the occurrence reason, demonstrate these conditions of industrial aggrega x :- of Black-water Fever amongst them.

As it is in the Duars that Black-water Fever is most evalent, this district will serve us best for the closer study of the conditions und which Black-water Fever occurs.

Spl rat

The Duars.

The district known as the Duars lies along the foot o er the Eastern Himalayas between Nepal and Assam. To the west, and for all p actical purposes a mere extension of the Duars, is the Darjeeling Terai.

The rainfall in the Duars is very heavy, averaging ab ut one hundred and fifty inches in the year, and this, together with the con guration of the land which abuts immediately upon the foot of the mountai is very favourable to the spread of malaria. When first taken over by t e British about thirty years ago, the Duars was mainly unbroken forest; it owe its recent importance entirely to the tea industry, in connection with which the e are in the district some 250 Europeans, a somewhat greater number of Babus, a d an immigrant popula-

tion of about 150,000 coolies. The labour throughout the Duars is obtained under the "voluntary system," a system entailing, as we have seen, a more unsettled population and a more constant influx of immigrants into the district than the contract system; and the constant movements of the population and the continual influx of new-comers have led in the Duars to such widespread prevalence of malaria as probably does not exist over a like area anywhere else in India.

The most obvious special feature in the Duars is then the prevalence and intensity of malaria infection. If it were not for malaria, the Duars, for a tropical country, would be unusually salubrious. There is a cool season lasting several months and the hot season is, on account of the heavy monsoon, much cooler and less [illegible] than it is in Calcutta, Bombay, and Madras. The Europeans live [illegible] condition[illegible] healthy life and are not unduly exposed to fatigue or hardship. Bla[illegible]ratively good; and not lacking so far as we can see in any essential pa[illegible]ars. Yet Europeans in the Duars die from Black-water Fever, whilst those [illegible] Calcutta and the plains of India, broadly speaking, do not. In what respect[illegible] the conditions different? If diet were at fault the disease should be as common o[illegible] healthy gardens of Assam as among the planters of the Duars; nor should Bl[illegible] water Fever occur among the Babus, who do not modify their diet with a change of district. If exposure to the sun be a cause, the disease should not appear as it does among Babus engaged entirely in office work. That the mere moisture of the atmosphere unaccompanied as it is by great heat should produce such [illegible]th effect is inconceivable.

An idea of the [illegible]niversal prevalence of malarial infection may be gathered from the following lis[illegible] of garden lines showing the endemic index:—

Gardens.		Race.	Month.	Number of children.	Spleen rate.	Parasite rate.
			Dam Dim District.		Per cent.	Per cent.
Ellenbari	*a*	Paharias	September	20	95	75
	b	Madeshis	September	46	80	87
Washabari		...	October	50	78	60
Bagracote		...	September	68	67	72
Phulbari		...	August	66	92	62
Pathajora		...	October	40	78	65
Manabari		...	October	30	90	83

Gardens.	Race.	Month.	Number of children.	Spleen rate.	Parasite rate.
		Dam Dim District.		Per cent.	Per cent.
Ranacherra	...	October	100	89	82
Rungamutty	...	August	69	90	7
Meen Glas	...	August	27	93	78
Bungalow lines	...	August	33	91	91
Factory lines					
Butabari	...	August	28	5	75
Dewars lines	...	August	25	92	96
Narkati	...	October	50	93	96
				100	
		Low-lying gardens.		83	
Bullabari	...	October	10	89	86
Moneys Hope	...	August	26	92	85
Sissibari	...	September	40	93	90
Hati-jungle					
Barons	...	October	14	89	98
Hazodcherra					
Jamadars	...	October	9	90	94
North Grant	Coles and Santals	October	28	82	93
Hyapathia	...	January	25	96	88
		Chelsa District.			
Chalouni (a)	Paharias	August	25	72	52
Chalouni (b)	Chota Nagpuries	August	25	72	52
Chalouni	Santals	...	25	...	...
Sam Sing	...	December	40	55	35
Kilcot	...	December	44	95	83
Indong	...	February	16	87	87
		Nagrakata District.			
Luksan	...	August	17	99	82

Gardens.	Race.	Month.	Number of children.	Spleen rate.	Parasite rate.
				Per cent.	Per cent.
		Dinatorsa District.			
Jiniguri	...	November . .	53	..	70
Hattapara	...	April . . .	33	...	70
Lankapara	...	April . . .	..	50	20
Dulsinpara	...	May . . .	...	75	60
		Low-lying gardens.			
Huldabari	...	November	34	85	94
Telepara	...	November	112	95	89
Gayakuri	...	November	96	93	85
Hindupara	...	November . .	25	100	96
Gundapara	...	November . .	108	83	73
Lakhubari	...	November .	34	91	94
		Torsa District.			
Jaigon	...	May . . .	40	75	60
Jalpaiguri Town . .	...	September . .	20	40	25
Nayabusti	...	September . .	36	61	36

The rains which are very heavy fall from May or June to the end of September. For several months after the rains there are everywhere small trickling streams fed by springs and these swarm with the larvæ of *M. listoni* and some other species. By January most of the streams have become dry, and from this time onwards to April or May extremely few breeding places exist except on certain low-lying gardens where the springs are perennial. Anopheles adults are notably scarce after January, except in these latter situations, and during the months of February, March, and April they are rarely seen in bungalows; they are also extremely difficult to detect in the coolie houses. During this time, that is, from January to May, fever among Europeans is at its minimum; servants also complain very little of fever; and an examination of children's blood shows scantier infections than one finds later in the year, though the actual percentage of children infected remains much the same.

Very early in the rains malaria becomes more prominent than it has been: servants continually absent themselves on this account; and Europeans begin to

suffer severely. In August, September, and October malarial conditions are at their height, but undergo some slight diminution as the colder weather approaches, though up to January anopheles can be found without difficulty and cases of fever are frequent.

What applies to variations of malaria from effect of the season is equally applicable to locality. While on almost all gardens the endemic index is approximately 100 per cent. there are some where the conditions favouring the spread of malaria are much greater than others, so that Europeans and Babus are more subjected to infection and coolies themselves suffer more from malaria. The most noticeably malarious gardens are those so situated that throughout the whole year they are never free from the soakage of water from springs. Instances of such gardens are—Barons, Neora Nuddy, Baradighi, and Telepara, in all of which the whole garden areas are intersected by innumerable streams and swampy hollows with running springs; it will be seen from the list of endemic indices that the rate on these gardens is more than usually high. *ow.*—

Malaria among Europeans and Bengalis.—Though the European in the Duars is generally to all appearances in good health, enquiry will often show that he is constantly getting what he calls "touch of fever," and even if he repudiates ever getting fever, it will be found that he is subject to "goes of liver" or "bile," which are but the interpretation he puts upon the symptoms of a malarial attack. Among some sixty persons resident in the Dam Dim district to our knowledge over fifty have suffered from attacks of Blayer (malaria) during the year. In many cases the attacks have been very mild, probably because quinine is now being very generally used as a prophylactic, some forty-five out of the sixty persons having made more or less systematic use of this drug since May 1907.

The parasite most frequently found is the malignant tertian, although among native children the proportion of different forms of parasite is about equal. Among effects of malaria in Europeans may be noted the common presence of anæmia at the end of the rainy season, a large number of observations having shown that at this time of the year the average hæmoglobin percentage is about 85 per cent. of the normal; some four or five months later at the end of the dry weather it approximates, however, to 100 per cent. Enlargement of the spleen was found in five out of thirty-two residents in the Dam Dim district.

The Bengali Babus are still more subject to malaria. They live in houses that are generally situated in the very midst of the tea-house lines; and they and their families suffer often in a terrible way from malaria. So much so is this the case that it is customary for Babus to leave their families in their own country or to have them up for a few months at a time only; and the explanation invariably given is that ill-health makes this necessary.

A liability of Europeans and Babus to pass through periods of more than usually frequent and severe spells of malaria, the attacks recurring every week or ten days for months together, is very commonly observed in the Duars. The explanation as a rule is comparatively simple and depends more than anything else on mere circumstance. While malaria can be contracted without difficulty in nearly every bungalow in the district, there are some bungalows where the conditions are so favourable to malaria that those who live in them are more than ordinarily exposed to infection on this account; but above and beyond this there are other circumstances concerned.

1. *The nature of the population of his servants' lines.*—It may happen that his servants are more or less immune and that they have few or no dependants; on the other hand, owing to pure circumstance he may have not only susceptible adults, but a swarm of young children in his servants' lines. When this happens a European who has stood the climate very well for years may enter upon a period of ill-health quite noticeable to himself and his friends.

2. *The constitution of the labour on the lines near his bungalow.*—With a large influx of susceptible coolies there may result in any particular season or succession of seasons what is known as an "unhealthy year for coolies." If a European is so situated as to be much exposed to infection from his coolie lines, he will probably suffer along with his servants from the general exhalation of infection.

Liability to infection.—A factor which we must emphasise, since it is to our minds one of the conditions always associated with Black-water Fever countries, is that, quite apart fram the amount of malaria any resident may appear to be suffering from, he is exposed almost daily to inoculation with sporozoits. Anopheles, especially the small dark *M. Listoni* and *M. Funestus*, the most notorious malaria carriers, are not easily detected within a mosquito net; they are also very persistent in gaining an entrance and they frequently bite by day. The result is that very few Europeans, even when they take precautions, altogether escape being bitten; the majority take no adequate precautions and must certainly become the prey of anopheles night after night. When it is realised that the salivary glands of one in every four anopheles may contain sporozoits (56), it becomes evident that the amount of fever suffered from is not a question of a man having received a certain number of infections each resulting in due course in an attack, but one of some interaction between the parasite and the tissues resulting from time to time in the mastery of the former. It is very noticeable that in infection with the malignant tertian parasite there is a period of immunity following an attack so that, however heavily and frequently infected a person may be, he exhibits actual fever only at recurring periods of a week, ten days, or a fortnight.

Constant infestation.—The condition in the communities we are studying therefore cannot be considered one of so many infections or separate attacks, but is a process of continuous infestation calling strongly to mind, if we substitute anopheles and malaria for ticks and piroplasma, what is recognised as occurring in the case of cattle living in a piroplasma country.

We append a letter in the form of a diary, received from a planter who was simply asked for particulars regarding his Black-water Fever attacks:

Arrived in India December 1894. Age 21.

1894-95. *South Sylhet*—

Constant attacks of malaria. Treatment 60 grains of quinine daily during attacks. No quinine taken between intervals of attack. No sign of anything approaching Black-water Fever.

1895-96. *Alipur-Duars*—

Constant attacks of malaria. Treatment daily doses of quinine 15 to 30 grains during and subsequent to attacks. No quinine between intervals. No sign of anything approaching Black-water Fever. Constipation general throughout the year and weight reduced from 12-5 to 11 stone.

1896-97. *Alipur-Duars*—

Constant attacks of malaria. Treatment 15 to 30 grain. quinine daily during and subsequent to attacks. No quinine in intervals. Malarial attacks culminated in June in bad attack of Black-water Fever. The disease raged for ten days and as life was despaired of I was carried to railway terminus and taken to Darjeeling. Began to recover rapidly and returned to garden six weeks later. Weight 10 stone

Quinine was taken in small lots of 10 to 30 grains on odd days just before the attack as I felt "seedy." Was treated for liver, dosed with mercury and black draught, and the day after developed Black-water Fever. Throughout the attack was given quinine and Tincture of Warburg—the former hypodermically when vomitting badly. Constipation bad throughout the year.

1897 to August 1898. *Alipur-Duars*—

Constant attacks of fever, with quinine treatment as above. Attacked by Black-water Fever in July. Treatment much as before. The disease considerably lighter than before. Shipped to Darjeeling five days after Black-water Fever developed and recovered rapidly. Civil Surgeon refused to allow me to return. Constipation bad throughout. Weight 11 stone.

August 1898 to April 1899. *Lucknow*—

Occasional slight attacks of fever and usual treatment. No sign of Black-water Fever and health very good. Constipation bad. Weight 12-6.

April 1898 to December 1899. *South Sylhet*—

Occasional attacks of malaria. Treatment as usual. Health very fair. Weight 11-12.

1899-1900. *Dum Dim Duars (Phoolbarrie)*—

Frequent attacks of malaria and usual treatment. In October a sudden and violent attack of Black-water Fever lasting two days only. Cut short by removal to Darjeeling where recovery was remarkably rapid.

Quinine was taken during the attack and before in the usual fitful fashion. Constipation as usual, treatment for this considerably more successful. Weight 11 to 11-8.

1900-01. *Phoolbarrie Duars*—

Frequent attacks of fever throughout the year and usual treatment. A sharp attack of Black-water Fever towards the end of December 1901. Perhaps the lightest of the five. Quinine treatment during attack I forget. Constipation answering to treatment. Fair health.

1901-02. *Phoolbarrie Duars*—

Usual attacks of fever and usual treatment with quinine. Black-water Fever suddenly developed in the end of September 1902 and though not virulent it lasted quite seven days that is of actual Black-water. Dr. Brown was careful in regard to quinine and I think treated me with Cassia Beariana. Constipation same as last year. Weight 11st stone.

River trip made me perfectly right in two weeks.

1902 to May 1903. *Phoolbarrie*—

Fair health.

May 1903 to November 1903. *England on furlough*—

Took quinine regularly and no fever.

Note.—Up to 1903 all quinine treatment was with ordinary sulphate or bisulphate. From 1903 on, quinine hydrochloride only was used in S. C. Tabloids.

November 1903 to December 1904. *Kilcott Duars*—

Very careful to take 30 grains quinine and very little fever. Only fair health.

1904-05. *Phoolbarrie*—

Only two attacks of fever in the twelve months. Most regular in quinine treatment, 30 grains weekly. Constipation responding to treatment.

1905-06. *Phoolbarrie*—

I don't think I got any fever this year. If I did it must have been slight. Most regular with quinine. Constipation as above.

The doses of 15 grains each twice a week on consecutive days constituted a great strain mentally and physically. Doses of 5 grains *daily* passed entirely unnoticed.

1906-07. *Phoolbarrie*—

No fever. Most regular with quinine, but substituted 5 grains daily for 30 grains a week taken in 15 grain doses. Constipation as above.

1907 to July 1908. *Barrackpore*—

No fever. *Most regular* in quinine treatment.

The experience of this man as regards malaria is not that of a special case, but represents the life of a great many of the Europeans living in the more malarious parts of the tropics ; it is a good example of what we have come to look upon as life under Black-water Fever conditions.

No one who knows anything of India can confuse such a history with that of the life of the ordinary Englishman in the plains or great cities of India.

It is misleading in our opinion to cite from Black-water Fever countries cases of men who are supposed not to have suffered from malaria, or to speak even in this connection of men having been exposed to the "chance" of malarial infection, for we are here dealing with a *certainty* of frequent or even daily infection without possibility of escape except by the adoption of the most stringent precautions.

To sum up : we find in the Duars conditions which are already familiar to us as those prevailing in tropical Africa. The same conditions are seen in the Jeypore Agency and in just those parts of Assam where Black-water Fever has been shown by ourselves and others to occur. These conditions are not rightly defined as merely malarious. We know of several districts where there is malaria, but where the condition we have tried to picture is not present.

Chapter II.

Black-water Fever—Ætiological.

The first published record of Black-water Fever from the Duars were four cases described by Stephens and Christophers in 1900. But Dr. Brown, Medical Officer of the Dam Dim district, assures us that the disease was present when he first came out to the country in 1887, but that it was not then recognised as Black-water Fever. The first to name the condition in the Duars was Dr. Wheeler who had previously seen the disease on the west coast of Africa.

Owing to the kindness of Dr. Brown we have been able to secure records of the cases seen by him during his twenty years' residence in the Dam Dim district; and from Dr. McCutcheon and Dr. Stone we have obtained similar information, extending over the last ten and five years, respectively, in regard to the neighbouring Chelsa and Nagrakata districts.

This information we give in full in an appendix. By means of it we have been able to form conclusions as regards the seasonal incidence and local distribution of Black-water Fever not obtainable from a lesser number of cases; it also serves to show that Black-water Fever was present among Europeans as far back as 1888.

The increase in cases during the later years is probably only apparent: in the first place Dr. Brown had not, when first in the district, charge of the whole of the gardens; also a certain number of cases which have occurred during Dr. Brown's absence have been added by us, these being chiefly recent ones. Apart from this there is the important fact that everyone in the Duars now knows and dreads Black-water Fever, and it is doubtful if latterly any cases in Europeans have been overlooked, though they might have been ten or more years ago.

The influence of enquiry and recognition of the disease is well exemplified by those cases recorded among Babus. It will be noticed that there are but two cases in Babus in the years 1887—1898. In 1900 the Duars was visited by the members of the Royal Society's Commission on Malaria and two cases in Babus were seen by them in the space of a fortnight. More attention was then turned to such cases, it having been more or less taken for granted previously that the disease chiefly occurred among Europeans. Since this time there have been

one or more cases recorded every year. In 1907 public interest was much aroused in regard to Black-water Fever, with the result that a great increase in reported cases occurred. We see no grounds whatever for regarding these facts as indicating a real increase in frequency.

Racial incidence.

The Europeans in the Dam Dim district are about sixty in number. Among these in the years 1887—1907 forty-four attacks of Black-water Fever have been recorded. In the Chelsa district among forty Europeans there have been in the years 1898-1907 eight recorded attacks. These figures would give an apparent incidence of about 3 per cent. and 2 per cent. *per annum*, respectively, among Europeans in the district. But as previously noted older residents are but little liable to the disease, and an allowance has to be made for this. The number of cases recorded among Babus in the Dam Dim district has been twenty-four and in the Chelsa district thirteen: but since we have ourselves seen thirteen cases in these two districts in a little less than a year, it is evident that many cases have previously escaped observation. In the years 1907-2908 the incidence of Black-water Fever among the Babus in the Dam Dim district as shown by the cases seen by ourselves, has been about 10·2 per cent. *per annum*, while during the same period it has reached 15 per cent. in the Chelsa district. The incidence among Chinamen is also heavy, as we have seen two cases in little less than a year and the number of Chinamen resident in the Duars is not large. Among Mahomedan bearers, syces, and other servants we have seen several cases, but we can give no definite information as to the incidence rate, as the number of people of this class is unknown and they come and go at frequent intervals.

Black-water Fever seems to be less common among the coolie class than among the bungalow servants and other natives from the actual plains of Bengal; but we have seen three cases in coolies from Chota Nagpur, and the disease may be more frequent than is apparent, the difficulty in finding such cases under the labour conditions existing in the Duars being great. Native children from shortly after their birth are subject to continuous malarial infection, and it seems probable that the condition might occur among them at the age from six months to two or three years; among coolie children entering the Duars with their parents for the first time also the disease might be encountered, for many of them die in the first two years of residence: but so far in spite of a good deal of enquiry we have been unable to get satisfactory evidence of hæmoglobinuria among young coolie children.

Seasonal incidence.

Arranged in the months in which they occurred the cases of which we have been able to get particulars are —

Months.	Districts.		Total.
	Dam Dim.	Chelsa.	
February	4	1	
March	1	...	8
April	2	...	
May	4	2	
June	3	1	16
July	5	1	
August	6	2	
September	8	5	34
October	10	3	
November	5	1	
December	6	4	25
January	8	1	

In both the Dam Dim district and the Chelsa district therefore the months that are healthy as regards malaria are notable for the low incidence of Black-water Fever cases; whilst in those at the latter end of the year when malaria is rife the incidence is much higher.

Our own cases seen between August 1907 and July 1908 have occurred as follows :--

August	6	
September	4	13
October	3	
November	1	
December		5
January	2	
February	1	
March	...	3
April	2	
May	1	
June	2	6
July	3	

A closer study of these cases shews up the relation to malarial incidence still more strikingly. The thirteen cases in August, September and October were distributed over the gardens Chengmari, Meen Glas, Chalouni, Nagasuri, Bagracot, Ranicherra, Monabari, Pathajora and Nagrakata, all gardens situated on high ground, which at this time of the year having many running streams is very suitable for malaria.

The cases in November, December and January occurred, one case at Jalpaiguri, the other four in the gardens Baradigi, Bentguri and Hahiapathia, all low-lying gardens noticeable as still having much water when the upper gardens were mainly, and in some cases entirely, dry. Meen Glas, for instance, which gave us 3 cases in August when it was intersected by innumerable small streams, was found by us in January to be free from water.

Of the three cases in February, March and April, two in April occurred in low-lying gardens, Baridighi and Ellenbari, notable as gardens where breeding places still existed when over most of the district they had long dried up.

The case in May just before the onset of the rains was on a low-lying garden (Huldibari) with perennial streams. Four cases in June and July were on upper gardens (Mateli, Indong and Monabari) and occurred after the onset of the rains, at a time when anopheles on these gardens were in great profusion and the fever season had commenced. The remaining case was in a European who had a slight attack in May, and a recurrence in June, after returning (from

a sea trip) to the low-lying garden on which he had contracted the disease. These are remarkable examples of the close association of malaria and Black-water Fever, showing that the occurrence of attacks of the latter bear a close relation to the incidence of the former.

Local incidence.

This may be shown by a table in which the cases have been arranged according to the gardens on which they have occurred—

Dam Dim District.

Gardens.	No. of residents.		Cases of Black-water Fever.			Figure representing liability to attack among Europeans.
	European.	Babus.	European.	Babus.	Others.	
Neora Nuddy	1	3	3	1	1	3
Barons	4	4	7	5	...	1·75
Sissibari	2	3	3	1	...	1·5
Bullabari	3	3	4	2	1	1·3
Choolbari	3	3	2	...	...	·75
Glencoe	3	3	2	...	...	·75
Meen Glas	4	4	2	2	1	·5
Bagracote	2	3	1	2	...	·5
Manabari	2	3	1	2	...	·5
Sungachi	2	3	1	...	...	·5
Toonbari	2	2	1	1	...	·5
Pathajora	2	3	1	...	1	·5
Ranacherra	3	3	1	...	1	·3
Ellenbari	3	3	1	...	...	·3
Bentguri	2	3	...	...	1	...
Washabari	1	3	...	...	1	...
Rungamuttee	6	5	...	1	4	...
Hahiapathia	2	3	...	2	...	...
Sylee	2	3	...	...	...	...
Needem	1	2	...	...	...	...
Narkati	2	3	...	...	...	...
Kumli	2	3	...	...	...	...

Chelsa District.

Gardens.	No. of residents.		Cases of Black-water Fever.			Figure representing liability to attack among Europeans.
	European.	Babus.	European.	Babus.	Others.	
Engo	1	2	1	...	...	1
Nagasuri	3	3	2	4	1	·67
Chelsa	6	4	2	...	...	·3
Sathkaya	3	2	1	...	...	·3
Kilkote	2	2	...	1	...	...
Mitale	4	3	1	...	...	·25
Baradighi	...	4	...	2	...	...
Moortee	2	2	...	...	1	...
Chalouni	3	3	...	1	2	...
Aibeel	2	3	...	...	...	...
Sam Sing	4	4	...	...	...	...
Yang Tong	2	2	...	...	...	...
Indong	3	3	...	...	...	...
Kumal	3	3	...	...	...	...
Zeranti	2	3	...	...	...	...

Our own cases have occurred as follows :—

Dam Dim.		Chelsa.		Nagrakata.		Dina Torsa.	
Meen Glas	3	Baradighi	4	Chengmari	1	Huldibari	1
Manabari	2	Nagasuri	3	Nagrakata	1	Tussati	1
Bagracote	1	Chalouni	1	.....		.....	
Ranacherra		Moortee	1	.....		.....	
Pathajora	1	Indong	1	.....		.....	
Hahiapathia	1	.....		.....		.....	
Bentguri	1	.....		.....		.....	
Ellenbari	1	.....		.....		.....	

Before deducing the relative liability of persons on these different gardens to attack, it is necessary to consider in each case the number of residents concerned and also to separate from among the number of attacks those belonging to Europeans and Babus, respectively.

The number of Europeans on a garden in any given year varies very little, and we give the number known to us at the present time, which may be taken to represent fairly accurately the numbers exposed to Black-water Fever on those particular gardens. The cases among Europeans probably represent the actual incidence more or less accurately ; among the Babus, as we have remarked before, the number of cases reported is probably only a portion of those actually occurring, and this can only be relied upon as a very rough indication of the incidence for 1898—1907. The figures for 1907-08, on the other hand, include most, if not all, the cases that have occurred, and give a much more accurate indication of places where Babus are most liable to attack.

The gardens thus prominently brought forward are :—

Neora Nuddy.—This garden is situated further south than any of the gardens of the Dam Dim district. Being a lower-level garden it is correspondingly more waterlogged. It is situated amongst a number of old beds formed by the constantly changing courses of the Chel and the Neora rivers, at the junction of which the garden is situated, and in the months of February, March and April, when the gardens nearer the hills are practically devoid of water many pools still exist at Neora Nuddy. One European lives at the garden, which is comparatively small. In all cases the Europeans have suffered very severely from malaria, so that the garden has an extremely bad reputation.

Barons.—This garden is one of three or four, including Bullabari, Sissibari, Kumli, Glencoe and Hahiapathia situated just where the drainage from the higher land issues in a line of springs and gives rise to innumerable trickling streams and to swamps, many of which contain water throughout the whole year. The streams which swarm with larvæ of *M. Listoni* are so numerous that it is scarcely possible to walk more than one hundred yards without crossing one of them.

The assistants' bungalow, where most of the European cases have occurred, is surrounded on three sides by a deep hollow containing a small stream, which only disappears for a month or two at the end of the dry season. Within one hundred yards is a larger stream running throughout the year. This stream contains much vegetation, and anopheles larvæ swarm in it. At a slightly greater distance is a small swamp. Within one hundred yards of the bungalow are extensive coolie lines. In no other bungalow in the Dam Dim or Chelsa districts are the conditions that favour malaria so striking and pronounced.

Bullabari.—Adjoins Barons: but is on a slightly higher level though almost as intensely malarious.

Sissibari.—Also adjoins Barons; and is under somewhat similar conditions as regards malaria.

Meen Glas.—Is a garden at the immediate foot of the hills, adjoining uncleared forest. In the dry season there is practically no water except that obtained from deep wells, but in the rains it is crossed by numerous small shallow streams, which remain running until December or thereabouts.

Both the manager's and the assistants' bungalows are very badly situated as regards malaria, the latter being within a short distance of one of the streams mentioned above.

The following was the result of the examination of the blood of those living in the servants' quarters at the assistants' bungalow in August:—

	Spleen.	Parasites.
1. Child, 3 years	Spleen	Benign Tertian Rings.
2. „ 5 months	„	Benign and Malignant Tertian mixed.
3. Woman, 22 years	„	*Nil.*
4. „ 20 „	*Nil*	*Nil.*
5. Boy, 10 months	Spleen	Benign Tertian Rings and Gametes.
6. Woman, 25 years	*Nil*	*Nil.*
7. „ 20 „	Spleen	Malignant Tertian Parasites.
8. Child, 1 year	„	Crescents, also Benign and Malignant Tertian Rings.
9. Woman, 20 years	*Nil*	Benign Tertian Parasites.
10. Sweeper, 25 „	*Nil*	*Nil.*
11. Cook, 35 „	Very large spleen	*Nil.*
12. Mesalchi, 25 „	„ „	*Nil.*
13. Child, 18 months	Spleen	Benign Tertian Rings and large forms.
14. Girl, 4 years	Spleen	*Nil.*
15. Woman, 25 years	*Nil*	*Nil.*
16. „ 25 years	*Nil*	*Nil.*
17. Girl, 3 years	*Nil*	Quartan Parasites.
18. Woman, 28 years	*Nil*	*Nil.*
19. „ 50 „	Spleen	Benign Tertian Parasites.
20. Bearer, No. 1, 30 years	*Nil*	*Nil.*
21. Bearer, No. 2, 28 „	Spleen	*Nil.*
22. Syce, 35 years	*Nil*	*Nil.*
23. „ 30 „	*Nil*	*Nil.*
24. Grass-cutter, 14 years	Spleen	*Nil.*
25. „ 20 „	Spleen	*Nil.*

In this case all the actual bungalow servants (male adults) were getting 10 grains quinine once a week; but none of the women or children were doing so.

One of the two European residents in this bungalow had Malignant Tertian infection with Crescents.

Baradighi.—Occupies the position in the Chelsa district that Neora Nuddy does in the Dam Dim district, being a good deal further away from the hills, and at a lower level, than any other garden in the district. It is situated close to various old beds of the Neora river, and is cut up by swampy hollows and flowing streams. Everywhere there is tall jungle and dense swampy growth, the general effect being more like conditions in tropical Africa than any other place we have seen in India.

Two of our cases occurred in the Babus' houses, which are situated close together; one in the bunya's shop, and one in the tea-house lines: all of which places are within a hundred yards of each other. A fifth case, which we were unable to see, has since occurred. We first visited this garden in December getting word of two cases which had occurred almost simultaneously. On reaching the garden, we found a third Babu, who had been in the garden only a fortnight, down with an ordinary attack of simple tertian, in the bunya's shop, where the third case later occurred, we found two out of three of the shopkeepers with parasites in their blood, it being one of these two who was subsequently attacked with Black-water Fever.

Without exception, the gardens on which Black-water Fever is more than usually frequent are those which one would point out as the most malarious.

Effect of residence.

Of the 160 cases recorded by us, in which we have the necessary information, there is only one which may have occurred under six months' residence; this was in a Bengali child, who contracted Black-water Fever after about three months' residence at Barons, under conditions of the most *intense malaria*. In Europeans there have been no cases under six months' residence, and considering first attacks in Europeans, where exact data is available, we get the following results:—

	Attacks.
Under 6 months	0
Between 6 and 12 months	8
„ 12 „ 24 „	40
„ 24 „ 36 „	12
„ 36 „ 48 „	5
„ 48 „ 60 „	1

Examining in detail the cases on the fourth and fifth year, we find that, in both cases, the man had been for a period of about two years under new conditions ;as for example :—

Case No. 4.—One year at Narkati
Two years at Rangamutee } Both as regards Europeans comparatively healthy gardens.

Then went to Barons, and was attacked two years after going there.

The same tendency to bring residence under Black-water Fever conditions to about two years is noticeable even in those cases in the third year.

Case No. 5.—Kumlai ;
then to Barons where he was attacked 18 months later.

In regard to the occurrence of attacks after five years' residence we have in Europeans :—

Cases which have occurred during the first five years . . .	24
Cases which have occurred at some later date	5

Of these five cases at later dates, we find that two had been home and came out eight months and two years respectively before attack : the history of one of the other cases is characteristic.

Case No. 51.—Had been out 10 years. Went to Neora Nuddy in December 1901. Went home and came out end of 1902. Stayed at Neora Nuddy and had fever. In 1905 went to Sungachi (Sungachi bungalow is very well situated as regards malaria) and there had the healthiest year he had had in the district. In 1906 went to Neora Nuddy and suffered badly from fever; he describes it as the worst time he had in the Duars with attacks of fever repeated at shorter and shorter intervals until Black-water Fever intervened.

In Babus the same tendency for first attacks to occur after about 18 months or two years' residence is noticeable ; but a large number of cases occur in Babus who have been many years in the country. The reason for this is not very clear ; it is possible that closer acquaintance with the conditions which influence this community might throw light on the matter. In many cases we have noted a special period of infection preceding attacks, suggesting that for some reason the conditions under which a Babu had been living had become changed (*vide* Case XI).

After the first attack there seems a tendency for further attacks to occur within a few months. Whether this is due merely to a continuance of the condition or argues an induced greater susceptibility is uncertain. A summary of the periods at which second attacks occurred in our cases shows that there is

the greatest liability during the first year, and especially the first six months, after an initial attack :—

1st month	0
2nd ,,	1
3rd ,,	2
4th ,,	4
5th ,,	1
Between sixth month and end of first year	4
Between first and second year	2
Over second year	3

Third attacks are in the Duars much less frequent than second attacks.

That residence for a certain time under special conditions is necessary before Black-water Fever can be contracted, and that after several years there is a greatly reduced likelihood of attack, cannot we shall see later be explained as being due to mere accidental circumstances; and, if we decide that this relation to length of residence is bound up with the essential nature of Black-water Fever its recognition must profoundly modify our conception of this disease.

Coolie Anæmia.

An extremely interesting condition is we think worth recording. Planters in Assam have long recognised that a large proportion of new coolies, especially at the end of their first season, and during the whole of their second and sometimes their third year, are particularly liable to suffer from anæmia of the severest type. This tendency is most marked among certain classes, notably North-Westers, Central Province coolies, Ganjamis, and people from the plains of Bengal; coolies from Chota Nagpur, the Santhal Pargannas, and the hill tracts of Madras do not suffer so frequently. The period from the second to the third season is extremely fatal for new arrivals, but once this dangerous time is passed and recovery from anæmia has taken place, acclimatization becomes more or less complete, and by the end of the fourth year a coolie is far less liable to suffer from sickness than he was on first arrival. A similar condition may be recognised among coolies coming to the Duars. There is not the least doubt that this condition is due almost entirely to malaria, and the length of the period required for immunisation is very significant in relation to what we have noted of the occurrence of Black-water Fever in Europeans.

Special Susceptibility and Favouring Conditions.

It is our experience that those who have suffered more than usual from malaria in a Black-water Fever country fall victims in a proportionately short period to Black-water Fever.

The following are the cases in Dr. Brown's records in which attacks of Black-water Fever occurred one year or under from first arrival. A perusal of the cases in the complete list (*vide* Appendix) will show that Dr. Brown has especially picked these people out as suffering more severely than usual from malaria :—

Case 7—Mr. T.—Came out from home to *Barons*. After first few weeks suffered from repeated and severe malaria. Had Black-water Fever eight months after arrival.

Case 24—Mrs. G.—Suffered very badly from malaria, and was unable or unwilling to take quinine on account of pregnancy. Had Black-water Fever six months after arrival.

Case 58— Mr. T. (*Padre*).—Notoriously exposed himself to infection in the discharge of his duties, and suffered more than usual from constant attacks of fever. Had Black-water Fever one year after arrival.

Case 9—Babu S.—Came with his wife and daughter, aged 8 years, to live at *Barons*, North Grant, in the midst of coolie lines situated in the most malarious conditions of which it is possible to conceive. A few months after arrival (September) the daughter was attacked with Black-water Fever, but recovered. In February following, 9 months after arrival, the wife was attacked with Black-water Fever and died. In June, a year after arrival, the Babu was attacked, but recovered. In December the daughter was again attacked and died.

Case 49—Mr. P.—A Frenchman from Pondicherry. Lived at Neora Nuddy and suffered a great deal from fever. Was attacked with Black-water Fever after eight months.

The same association with malaria also applies to questions of change of residence, of season, and of locality, to the existence of Black-water Fever houses, and the special susceptibility of relations, and of persons living in the same place, to incur attacks of Black-water Fever; for such cases seem always most simply explained by the amount of malarial infection that has been experienced.

Whatever explanation be given it still remains a significant fact that, not only is the general distribution of Black-water Fever and malaria in India closely related, but the investigation in detail of such a district as the Duars shows that Black-water Fever is most frequent on the most malarious gardens, and in the most malarious seasons; also that it tends to occur early and to be severe in the case of those who have been especially subject to malaria.

The more minutely we enquire into conditions we find therefore not discrepancies, but an even closer relation between the incidence of Black-water Fever and the intensity of malaria; and this is so marked that one is forced to believe that, even if the diseases possess a different origin, they must none the less be dependent on almost similar conditions for their propagation.

That any circumstances of a general nature are likely to be concerned in the causation of Black-water Fever we have seen to be improbable; and we are left therefore with the alternative hypotheses :—

(1) That Black-water Fever is due to a specific organism.
(2) That it is of malarial origin.

Chapter III.

Black-water Fever not due to a special parasite.

Infectivity of Black-water Fever.—There is nothing in the general clinical picture of Black-water Fever to suggest that it is an infectious disease, such as Yellow Fever, or Spirillosis; epidemics have, it is true, been described, but with a want of accurate and detailed observation, which renders it impossible to judge whether hey have been merely the result of large numbers of persons being placed simultaneously under Black-water Fever conditions, or due to the spread of infection.

If malaria will produce Black-water Fever in 50 per cent. of the new-comers in a small community, there is no reason why in the case of a large number of susceptible persons brought more or less simultaneously under Black-water Fever conditions, there should not result a large number of cases simulating an epidemic. To quote, without particulars, epidemics of the disease on railways or in armies has therefore little bearing upon the question.

In the Duars attacks occur quite independently of one another in widely separated gardens and districts. The incidence, as we have pointed out, shows a frequency directly relative to that of malaria; and we have seen that attacks are more frequent in certain bungalows, in which there is especial danger of contracting the disease. In such bungalows, attacks occur generally at long and irregular intervals; but in regard to length of residence of those attacked they conform to the general rule.

Histories.

Neora Nuddy.—A bungalow with rarely more than one European.

P. attacked September 1897, after 8 months' residence.

L. attacked October 1905, after 7 months' residence; second attack in December 1905.

S. attacked January 1907, after one year's residence.

Note the early onset of the disease under especially intense malarial conditions.

Barons—

(*a*) Assistants' bungalow. Two men resident.

Mr. C., attacked May 1889 . . . 2 years' resident.
second attack, September 1889.

Mr. McL., attacked August 1890 . . 2 „ „

Mr. E., attacked October 1891 . . . 2 „ „
second attack, March 1892

Mr. D., attacked October 1899 . . . 2 „ „
second attack, February 1901.

Mr. G., attacked May 1901 . . 18 months' „

(*b*) Babu's house at North Grant lines. A single Babu and his family only resident.

Daughter, aged 8 years, attacked September 1903	3 months' resident.
Wife attacked February 1904	8 „ „
Babu attacked June 1904	1 year „
Daughter, second attack, December 1904.	

Note the sequence of child, woman, and man in the order of their susceptibility to malaria (*vide* page 44) and early onset under extremely intense malarious conditions.

Bullabari—

(*a*) Assistants' bungalow: Two men resident.

Mr. S., attacked January 1897	2 years' resident.
Mr. S., attacked February 1904	2 „ „
second attack January 1906.	

(*b*) Manager's bungalow (very close to last).

Mrs. G., attacked July 1896	6 months' resident.
Mr. G., attacked January 1904	Several years at bungalow, but returned from England two years before attack.

Sissibari—

Assistants' bungalow: One man resident.

Mr. T., attacked January 1889 . . .	Unknown.
Mr. W., attacked October 1901 . . .	2 years' resident.
Mr. F., attacked September 1906 . . .	18 months' „
second attack December 1906.	

The theory that one is dealing with accumulating chances of infection is scarcely a satisfactory explanation of such a state of affairs, for the infection if it exists is evidently most localised in the very houses where these people are living and it is inconceivable that, in spite of this, a new-comer should never, by any chance, be attacked.

And the conditions which we have just described are characteristic of Black-water Fever everywhere: in tropical Africa we have seen more than 50 per cent. of men who went to live at certain bungalows go down eventually with Black-water Fever; but in every case the peculiar feature of a certain length of residence was preserved.

What usually happens, when a new-comer comes to a house in which there has been much Black-water Fever, is that he very promptly contracts not Black-water Fever but malaria. Of this we have seen repeated examples.

Dr. Emlyn Jones (52) refers to a case where a nurse, who came from Calcutta to the Duars to nurse a Black-water Fever patient, was stated to have contracted Black-water Fever ten days later. On making enquiries of Dr. Brown he informed us that the nurse came to the Duars to attend a case of Black-water

Fever which was fatal in about ten days ; almost immediately after her return to Calcutta she had merely a severe attack of ordinary malaria. *

A new-comer who came from England direct to Barons in November 1907 contracted, not Black-water Fever, but severe malaria within ten days. Another person who came from Europe about the same time was attacked within the first month.

It is difficult to believe that Black-water Fever is an ordinary infective disease. If infective it must be present in some latent form ; and like malaria this must be endemic.

Possible Organisms concerned.—The special organism that would at first thought appear most probable as the cause of Black-water Fever is some form of piroplasma, since this is the only kind of parasite we know producing such symptoms.

Of piroplasms there are two groups broadly distinguishable :

(*a*) The large typical forms *P. Canis*, *P. Bigeminum*, and *P. Ovis* ; and the irreguler forms, more or less minute, lately classed in a new genus *Theileria*. Hæmoglobinuria, in so far as it has been recorded, is confined to the first group. It occurs in the dog (Malignant Jaundice), the ox (Red-water), and the sheep (Carceag).

In the ox and dog hæmoglobinuria is associated with a large number of parasites in the peripheral blood and in the organs. In infection with *P. equi* parasites are also present n large numbers. In our own experience, among dogs hæmoglobinuria never occurs except in connection with infections which it would be impossible for any competent observer to overlook.

East Coast Fever, a disease due to infection with *P. parvum*, one of the minute forms of piroplasma, is characterised by visceral lesions of a marked kind ; but hæmoglobinuria is not a symptom : and even the minutest orms of piroplasma, though their demonstration may require some care in the preparation of specimens are, if present, at once evident to critical examination.

Heart-water in sheep has been shown by Lounsbury (53) to be transmitted by ticks (Amblyomma) ; and it has on this account been suspected to be a piroplasmosis due to an ultra-microscopic parasite. But here again hæmoglobinuria is not a symptom ; and we have too insufficient a knowledge of the disease to base conclusions on the above mentioned view of its nature.

Judging from analogy, we should expect, if Black-water Fever were a piroplasmosis, to find parasites without difficulty in the peripheral blood and organs.

Some other parasites (Relapsing Fever, Yellow Fever, etc.), however, give rise to a good deal of blood destruction, and even hæmoglobinæmia. (But *vide*

* *Note.*—This was ascertained by personal enquiry.

extra-vascular lysæmia, Part III); we have therefore to bear in mind *Spirochætæ* or even *Bacteria*. Hæmoglobinuria is also described in certain fluke infections (Dantec) (54), and it is now recognised that in Anchylostomiasis the anæmia may possibly be due to the action of hæmolytic bodies produced by the worms: there is thus considerable scope in the possible parasitic conditions that may give rise to Black-water Fever. But it is the analogy of the parasitic hæmoglobinurias of animals, which has chiefly influenced conceptions of the disease; and if such an analogy is found not to hold good, there is a greatly diminished probability of Black-water Fever being a disease of specific origin.

Examination of the Peripheral Blood.

In these observations we used very carefully-made films stained by Giemsa's stain, with and wihout the addition of a trace of alkali, which greatly enhances its staining power. Our lenses were Zeiss apochr. f. =1·50 N. A. =1·30 with compensation eye-pieces No. 6 and 12.

But the most minute and exhaustive scrutiny of the red cells, the plasma and the leucocytes of our cases has failed to give any indication of the presence of a piroplasma-like organism; yet several of our cases have been seen quite early in the course of the attack, and two only a few hours after the onset.

The use of neutral red in two cases, one of which was a severe one with intense hæmoglobinuria, has shown corpuscles stained in a characteristic manner (*vide* Spherocytes, Part III): but the closest scrutiny showed no included parasites.

Examination of Splenic Blood.

In all cases where it was possible we obtained splenic blood by puncture during life within a few minutes of first seeing the patient. The particulars of these observations are as follows:—

Case XIX.—An extremely mild case. Spleen punctured shortly after onset of hæmoglobinuria (8 to 10 hours) and shortly before its cessation.

Case XX.—A severe case. Spleen punctured about 4½ hours after onset and 48 hours before hæmoglobinuria ceased.

Case XXII.—A severe case seen 30 hours after onset. Spleen punctured while hæmoglobinuria still continued.

Case XXIV.—A severe case seen on fourth day just before the hæmoglobinuria ceased.

Case XXVI.—A severe case seen on 2nd day and about 36 hours before hæmoglobinuria ceased.

Further details regarding these cases are given in Part III and in the Appendix.

The same minute attention was given to these films as we gave to those of the peripheral blood. The red cells, the intervening spaces, the leucocytes and macrophages with their inclusions were in turn scrutinised, *several days being devoted to the examination of a single film.*

Beyond such malarial parasites as are recorded later, and the presence of malarial pigment, we failed to discover any foreign body. On the other hand, we encountered large numbers of red cells engulfed in macrophages and leucocytes many of which were absolutely normal in appearance; and none of which showed any contained parasite.

Observations with regard to the possibility that a Black-water Fever Parasite has been mistaken for the Malarial Parasite.—In experiments among pariah dogs with *P. canis* we frequently encountered infections which did not give rise to hæmoglobinuria; we had also been struck by the fact of the occasional quite decided, but superficial, resemblance of piroplasma to certain young forms of the malarial parasite. The contention that special parasites had been sometimes seen but mistaken for malarial parasites seemed to us therefore to have some slight possible foundation.

It was therefore necessary not only to carefully scrutinise any apparent malarial parasite seen in Black-water Fever; but also to ascertain if any parasite not malarial occurred in the blood of persons living under Black-water Fever conditions, or in the natives living around them. In order to fit ourselves for this we studied very closely indeed the younger forms of the malarial parasites, and especially the malignant tertian variety; for the more advanced forms of simple tertian and quartan it was impossible to consider anything but what they seemed. The result of this study we hope to publish later.

It is sufficient for the present to state that our own cases of Black-water Fever have shown typical examples of ordinary malarial parasites and in most of our cases recent malarial pigment was present. These observations are in accord with the results obtained by many other workers in whose recorded cases the presence of malarial pigment seems to preclude the idea of the parasite being other than malarial.

The study of a large number of blood specimens taken from the adults and young children living in the immediate neighbourhood of the houses in which Black-water Fever cases had just occurred likewise failed to demonstrate the existence of any but undoubted malarial parasites.

The results of a number of such examinations are recorded below :—

BARADIGHI.

Babus	Babu I	Black-water Fever.
	„ II	„ „
	„ III	Simple Tertian parasites.
Bunyas	Male, 17 years	Quartan parasites. Late had Black-water Fever.
	„ 25 „	No parasites.
	„ 20 „	Simple Tertian parasites.
Children in small groups of huts near Babus' houses.	Group I	3 years Simple Tertian.
		10 months „ „
		4 years „ „
		2 „ „ „
		4 „ „ „
	Group II	6 months „ „
		4 years . Simple Tertian and crescents.
		3 „ . Malignant Tertian and crescents.
		1 year . Simple Tertian and crescents.
		3 years . Simple Tertian.
	Group III	Male, 45 years *Nil.*
		„ 25 „ *Nil.*
		Boy, 12 „ *Nil.*
Children in Santal lines near Babus' houses and bunya's shop.		2 years . Simple Tertian and Quartan.
		4 months . Simple Tertian.
		8 „ . Malignant Tertian swarming.
		6 „ . Simple Tertian.
		2 years . „ „ and Quartan.
		5 „ . „ „
		2 „ . „ „
		7 „ . *Nil.*
		3 months . Simple Tertian and crescents.
		1 year . Quartan.
		2 years . Simple Tertian.
		1 year . Quartan.
		2 years . Simple Tertian.
		Woman, 30 years . Pigmented leucocytes and single small ring.
		Man, 20 years *Nil.*

Children in Nagpuri lines near bunya's shop.	1 year	. Simple Tertian and crescents.
	7 years	. Simple Tertian.
	5 „	. Quartan.
	6 „	. Simple Tertian.
Servants' lines near bunya's shop	Boy, 18 years	. Quartan.
	„ 1 year	. Simple Tertian.
	„ 2 years	. „ „
	„ 8 „	. *Nil*.

BARONS.

Lines near assistants' bungalow	5 years	. Simple Tertian.
	1 year	. *Nil* (but enlarged spleen and anæmic).
	2 years	. Simple Tertian.
	3 „	. Quartan.
	6 „	. „
	10 „	. Simple Tertian.
	2 „	. Quartan.
	5 „	. Malignant Tertian rings.
	2 „	. *Nil* (new cooly).
	5 „	. Quartan.
	11 „	. Crescents.
	1 year	. Simple Tertian.
	3 years	. „ „
	3 „	. *Nil* (mononuclear increase).
	4 „	. Quartan.
	6 „	. Simple Tertian.
	6 „	. Quartan.

BENTGURI.

Manager's bungalow servants	Mohamedan	30 years, large spleen, no parasites.
	„	32 „ „ „ „
	Cooly class	18 „ no spleen „
	„	25 „ „ „
	Syce (Bengali)	enlarged spleen „
Assistants' bungalow servants	Mohamedan	32 years, Black-water Fever.
	„	20 „ Simple Tertian. Intensely anæmic.
	Syce (Bengali)	18 „ Simple Tertian. Intensely anæmic.
	Waterman	Malignant Tertian. Fever at times.

European. Aged 40. No parasites. Living at Manager's bungalow.

European. Aged 25. Anæmic. No parasites. Living until recently at assistants' bungalow.

The examination also of a number of Babus living under Black-water Fever conditions, the results of which are given a little later, showed the presence of none but typical malarial parasites.

These observations appear to us to exclude the possibility that Black-water Fever is due to a human piroplasmosis. They do not, as we are aware, absolutely preclude the existence of a special parasite in Black-water Fever; but they tend to show that the hypothesis which puts this view forward must confine itself to the suggestion that the disease is not an ordinary piroplasmosis, *or due to a blood parasite visible to critical microscopical examination.* With this modification of the specific disease theory we shall deal later.

CHAPTER IV.

SPECIFIC RELATION OF BLACK-WATER FEVER TO MALARIA.

It is sometimes stated in recorded cases of Black-water Fever that the patient had not previously suffered from malaria ; this statement, if true, is evidently a strong argument against Black-water Fever being the direct outcome of constant malarial attacks. Our own experience had led us to doubt very much whether such histories are trustworthy. For reasons already given we think it is very improbable that a man could live for several years in any Black-water Fever country known to us without contracting malaria ; the ordinary European does not take precautions, and infection with malaria is one of the conditions imposed by residence, which few, if any, can escape, but which shows itself more prominently in some than in others. This must be exceedingly clear to all who have experience of the countries in question.

But since the reasons on which we base this view do not seem to be generally recognised it is desirable to emphasize them.

There are three conditions tending to obscure the frequency of malarial attacks in a highly malarious country—

(1) Familiarity of the patient with attacks which cause him altogether to ignore their importance.
(2) Refusal under certain circumstances to acknowledge the disease.
(3) Non-recognition.

It frequently happens that when European residents in an intensely malarious country are questioned as to whether they have suffered from malaria or have had any recent attacks they give a denial to the direct question, but on more closely cross-questioning them they admit a history of repeated " bilious attacks," " headaches," " touches of the sun " or fits of retching, or dyspepsia.

Without going into further detail we may say that, in our experience of malarious countries, " biliousness," " retching," " vomiting," " headache," " touches of the sun," and even " influenza," and " colds," are not necessarily " masked " malaria, but as a rule the symptoms of typical though more or less mild attacks of the disease. In one case a medical man in Africa told one of us that he was feeling a " bit bilious "; on examination of his blood a considerable malignant tertian infection was found.

We could, were it advisable, multiply such instances by the score; though such a want of recognition we feel bound to state has not been a feature in the Duars, where several of the medical men use the microscope freely in diagnosis.

Our motive for entering into a somewhat long discussion on this point is to emphasise the fact that histories of Black-water Fever patients, who profess not to have suffered from malaria, are to be treated with grave caution.

In all of our cases we have every reason to believe that, prior to the Black-water Fever, the patient had for some considerable time been suffering from repeated malarial infection.

In the great majority there is very strong probability, as shown by the history, that an actual attack of malaria immediately preceded the Black-water Fever. This view was amply confirmed, wherever early examination of the patient was possible, as will be seen from the following tabular abstract of our cases :

—	Previous history.	Prodromata.	Under 24 hours of onset.	Between 24 and 48 hours of onset.	Third day and later.
Case I —Babu .	Had been getting fever for which he had been taking quinine.	.....	...	...	3rd day. Pigmented leucocytes.
Case II.—Coolie .	Was being treated for fever and spleen by Dr. Babu.	.....	...	...	Abundant recent malarial pigment in organs.
Case III.—Babu's servant.	Not known	Came to garden looking for work. Seen to be ill.	...	...	Abundant recent malarial pigment in organs.
Case IV.—Dhoby .	Dr. Babu makes special note of much fever dating back three months at least.	Complained of fever four or five days preceding the black-water.	A single malignant tertian ring. 48 pigmented leucocytes in a count of 500 cells.	No parasites. 87 pigmented leucocytes in count of 500 cells.	3rd day. Two or three pigmented leucocytes.
Case V.—Babu .	Had been getting fever and was being treated for this with insufficient doses of quinine.	Had fever 10 days previously; also fever 10 days after attack with malignant tertian parasites.	Malignant tertian parasites. Later in day much reduced in number.	No parasites.	
Case VI.—Bearer .	States he has had no fever this year, but was taking quinine (insufficient) to prevent fever. But his statement is contradicted by his master who says that he has had fever.	Slight fever for two days prior to black-water.	...	No parasites. 4 pigmented cells in count of 500.	

—	Previous history.	Prodromata.	Under 24 hours of onset.	Between 24 and 48 hours of onset.	Third day and later.
Case VII.—Babu	Had to go to Calcutta for a change on account of constant fever.	Was taken with fever in the train for which he took quinine.	...	...	Mononuclears 28·5 per cent.
Case VIII.—Babu	Had been getting fever . .	Had an attack before black-water. Fever for two days before attack.	Malignant tertian rings.	No parasites. Pigmented leucocytes.	
Case IX.—Chinaman	Has had two previous attacks fo black-water.	Had fever for about three days before attack.	Malignant tertian ring. Scanty pigmented cells.	...	
Case X.—Policeman	Six months on duty out in district. Himself, wife, and daughter all suffered much from malaria.	Fever a fortnight before attack. Evidently at the time he took quinine he thought he was coming in for fever.	...	Two pigmented leucocytes.	
Case XI.—Babu	Came to a new garden three months before. Ever since coming has suffered from constant and severe fever, not less than and an attack once a fortnight.	.....	No parasites. 5 pigmented cells in 500.	...	
Case XII.—Coolie	Not known	Had "bad fever" for which he was given quinine.	...	...	3rd day. No pigment. Mononuclears 24·6 per cent.
Case XIII—European child.	Fever frequent	.....	Quartan parasites.	...	
Case XIV.—Policeman	*Vide* Case X—History of constant fever.	.....	...	...	3rd day. No parasites. 4 pigmented leucocytes.

—	Previous history.	Prodromata.	Under 24 hours of onset.	Between 24 and 48 hours of onset.	Third day and later.
Case XV.—Babu	Wife and himself have been suffering from constant fever.	.....	...	...	4th day. No parasites. No pigment.
Case XVI.—European	Constant fever	.....	...	2nd or 3rd day (?). Mononuclears 17·7 per cent.	
Case XVII.—Babu	Doubtful previous attack of black-water.	Fever for three or four days before attack.	...	Pigmented leucocytes.	4th day. No pigment.
Case XVIII.—Babu.	Frequent fever	Slight fever for some days before. Of two other Babus on this garden one had black-water (Case XVII); the other, a new-comer, simple tertian fever.	..	...	5th day. No evidence on microscopical examination of malaria. (*Vide* spleen.)
Case XIX.—Servant	Living under extremely bad malarial conditions. Fellow-servants fever-stricken.	Was treated for fever a week before and had fever on the day before his attack which intermitted.	No microscopical evidence of malaria. His three fellow-servants all had malarial parasites in their blood.		
Case XX.—Babu	History of fever and three months ago had an attack of "liver" with yellowness of his eyes.	Was being treated for three days before attack for fever.	Abundant malarial pigment in splenic macrophages.		
Case XXI.—European	Living in house that even in the dry season was much infested with anopheles.	Had been seedy for a fortnight. States that he was well when he took the dose of quinine preceding his attack.	Mononuclears 20 per cent. No parasites. No pigment.		

—	Previous history.	Prodromata.	Under 24 hours of onset.	Between 24 and 48 hours of onset.	Third day and later.
Case XXII.—Shop-keeper.	Repeated attacks of fever during the whole of the nine months he has been in the Duars.	Feverish a fortnight before. A few days before attack had high fever which remitted. The fever came on again and he passed black-water.	Crescents in spleen and abundant recent pigment.		
Case XXIII.—Coolie.	Doubtful	Had fever! (temp. 104) day before black-water.	...	...	Seen only on 6th day. No microscopical evidence of malaria.
Case XXIV.—China-man.	Doubtful	History vague and uncertain .	...	...	No evidence of malaria in peripheral blood. Abundant malignant tertian pigment and a sporulating parasite in the spleen.
Case XXV . .	Repeated fever	.....	...	...	Seen 5th day. Films not yet examined.
Case XXVI . .	Repeated fever	.....	...	Abundant recent pigment in spleen. Peripheral blood not yet examined.	
Case XXVII . .	Repeated fever	.....	...	Films not yet examined.	

It is impossible to overlook the fact that in the majority of these cases malarial infection has been present, and that this has disappeared in the progress of the attack.* Of ten cases seen on the first day six showed actual parasites and two more showed abundant recent pigment. Of the seven observations on the second day, none showed parasites; but in six there were pigmented leucocytes. On the third day out of five observations three showed a few pigmented leucocytes, none showed parasites. Of five observations on the fourth, fifth and sixth days only one showed evidence of malaria and this on splenic puncture. In both of the cases, where we have been able to study the organs, abundant recent pigment was present in the macrophages and leucocytes of the spleen and ver, though parasites had then disappeared.

These observations do not stand alone; they reproduce very closely the relation shown by Stephens (55) in his summary of observations upon this point.

Stephens' tabulation is as follows :—

Observer.	Day before onset.		Day of attack.		Day after onset.	
	Total.	Positive.	Total.	Positive.	Total.	Positive.
A. Plehn	5	5	5	3	10	2
F. Plehn	0	0	21	18	10	3
Koch	5	5	8	6	6	1
Stephens and Christophers . .	1	1	9	2	16	0
Daniels	3	3	3	1	2	0
Panse	9	8	17	9	20	5
Total .	23	22	63	36	64	11
Percentage positive . .	95·6 per cent.		61·9 per cent.		17·1 per cent.	

Similarly treated a tabulation of our cases shows :—

——	Day before onset.		First day.		Second and third day.		Fourth to sixth day.	
	Total.	Positive.	Total.	Positive.	Total.	Positive.	Total.	Positive.
Parasites . .	*Nil*		10	6	7	0	10	1†
Percentage positive.	.....		60 per cent.		0 per cent.		10 per cent.	

* *Note.—Cases XXV—XXVIII* are not included as films have not yet been adequately examined.

† Splenic puncture, a single parasite.

	Day before onset.		First day.		Second and third day.		Fourth and sixth day.	
	Total.	Positive.	Total.	Positive.	Total.	Positive.	Total.	Positive.
Parasites and pigment	*Nil*		10	8	7	6	10	4
Percentage . .	.....		80 per cent.		85·7 per c		40 per cent.	

Classifying the parasite finds the preponderance of malignant tertian is very noticeable—

Malignant tertian	6
Benign tertian	0
Quartan	1

It is obvious that the significance of these observations depends upon whether such an amount of evidence for malaria would be found in any indiscriminately selected persons of the same race and class. Our experience of blood finds in adults opposes such a supposition.

The examination of forty-five Babus and their servants in August, September and October, the most malarious part of the year, gave as follows :—

Not infected	34
Benign tertian	5
Quartan	2
Malignant tertian	4

Total 24·4 per cent. infections.

Of five Babus examined in May and June, one showed malignant tertian infection.

The examination of sixteen Babus in January gave :—

Benign tertian	0
Quartan	0
Malignant tertian	1
Pigmented leucocytes	1
No evidence of malaria	14

Total 12·5 per cent. infections.

As we have already noted, Black-water Fever is frequently seen in Babus long resident in the Duars ; our cases have been as follows :—

Under five years	3
Five years or over	7
Ten years or over	3

Since therefore the length of residence has played but a small part in this incidence of Black-water Fever the series of cases of Black-water Fever in Babus is comparable with that of the series examined for malaria, quoted above.

We have therefore evidence of a—

(1) Greater incidence of malaria among Black-water Fever cases than the rest of the community.

(2) A special relation to the course of the attack shown by the disappearance of the signs of malaria during the progress of the disease.

Granting the relation of malarial infection to Black-water Fever, which seems to us established, there are but two explanations possible, either that the malaria has a causal relationship to the Black-water Fever, or that the attack of Black-water Fever, on the analogy of Red-water in Rinderpest, by lowering the resistance brings on an attack of malaria in a subject already containing parasites in his system. We can only judge of the relative probability of these alternatives. If Black-water Fever only induces a malarial attack it is difficult to see why the latter disease should always precede the former, and be already over before the morbid process which called it forth has disappeared. It is obvious also that the conclusions in regard to the malarial origin of Black-water Fever are not altogether dependent on the proof of the association of the disease with an attack of malaria, which is the point demonstrated by the facts just given. The conclusion that Black-water Fever has a malarial origin is largely bound up in considerations given by us in preceding chapters, questions of similar distribution and intimate association. That some cases should yield no evidence of malaria on microscopical examination, or even be unassociated with an attack, is no bar to the view that they have come about as the result of much antecedent malaria.

Chapter V.

Conclusions in regard to the factors engaged in bringing about Black-water Fever.

A consideration of the facts presented forces us to the conclusion that in Black-water Fever we are dealing not with a specific disease, but some stage in the progress of malarial infection long continued and constantly repeated. Stephens has already voiced this conception when he says that malarial infection displays its intensity in Black-water Fever, men dying in Africa not from malaria, but from the resulting Black-water Fever. This conception is also in harmony with the ideas suggested by Marchiafava and Bignami (56-*a*) in their description of malarial hæmoglobinuria.

That in Black-water Fever, though induced by malaria, we see a distinct morbid process, and not a mere malarial attack, is shown by the relation of the condition to malarial infection. It is impossible to discard the evidence proving this relation; and once this view, that in Black-water Fever we have a disease not malaria, but malarial in origin is admitted, every known fact in regard to it is embraced in the theory: a statement which cannot be made of any other hypothesis. As we have thus unhesitatingly decided in favour of the malarial origin of Black-water Fever, that is of its being the outcome of the malarial conditions pictured in our second chapter, it may be thought that we have done so in face of a certain number of objections. This we do not allow: for a careful consideraton of the facts regarding Black-water Fever show no real objections; but, on the contrary, the closer such apparent objections are studied the more obvious does the truth of the malaria view appear.

Numberless objections have been raised on the misconception that Black-water Fever is in some way an attack of malaria. Such, for example, as that differences in the clinical manifestations of the two diseases prove Black-water Fever not to be malarial: or that a disproportion between the number of malarial parasites and the hæmoglobinuria showed that the presence of the former bore no relation to the latter.

These objections are foreign to the point at issue, and apply only to theories of the disease no longer held by any recent worker on the subject.

The series of objections that relate to alleged want of coincidence between geographical distribution and seasonal incidence of Black-water Fever and malaria we have shown do not exist. It cannot be suggested that in recent researches too much has been taken for granted in relating Black-water Fever to malaria; the more pleasing solution would undoubtedly be the demonstration of a specific organism; but the trend of evidence is steadily in favour of a malarial, as against

a specific, origin, so that it seems to us reasonable to recognise the probability of its being after all malarial.

Before reaching our final conclusions regarding the remoter causes which bring about Black-water Fever, it is necessary to refer once more to the theory which maintains this disease to be of a specific nature.

We have seen that no piroplasma-like organism can be found, either in the peripheral or visceral blood ; though in the parasitic hæmoglobinurias of animals large numbers of these organisms are to be found in these situations : that nothing in the peripheral or splenic blood suggests that a minute form of piroplasma is concerned : that the examination of many hundreds of blood films from children and others within the endemic area, and more careful search in the case of numbers of people of the susceptible class, together with children and others living in direct association with Black-water Fever cases or immediately around their neighbourhood, has likewise failed to disclose the existence of new parasitic forms.

In the face of these facts those who still maintain the specific nature of Black-water Fever must do so apart from the analogy of piroplasmoses in animals, an analogy which fails at the critical point. It appears to us idle to discuss in detail the arguments for and against this contention. Those who assert Black-water Fever to be specific must be prepared to assume the existence of an organism distinct from the piroplasmata and a disease endemic like malaria but not directly infectious nor epidemic like Yellow Fever. It must require even under the most favourable conditions many months or several years' exposure to infection for its inception, and possess the seasonal and local incidence of malaria and a geographical distribution confined to countries and areas where intense malaria occurs ; its manifestations must be invariably associated with coincident malarial infection, and it must possess the power like malaria of lying dormant in the system and of asserting its presence in subjects who have left the endemic area ; its attacks must in the majority of cases bear a specific relation to quinine administration, and their prevention by the systematic use of quinine must be explained (*vide* Prophylaxis, Part V) ; immunity to its action must be acquired not by recovery from attacks, which instead seem to render the subject more susceptible, but be associated with the occurrence of immunity to malaria. It must in fact always exist side by side and be inseparable from malaria and yet be distinct.

The probabilities of such an hypothesis being the true explanation appear to us to be extremely remote.

Part III.

BLACK-WATER FEVER DUE TO THE ACTION OF A SPECIAL HÆMOLYSIN.

Chapter I.

Clinical and Pathological Observations bearing upon the nature of the process concerned in Black-water Fever.

The features of an attack of Black-water Fever have been broadly outlined in the introduction ; but it is necessary to consider the disease in greater detail.

I.—Symptomatology.

Variations in the general character of an attack.—An attack of Black-water Fever as a rule pursues a very definite course, the hæmoglobinuria usually lasting two or three days. But, especially in the case of Bengali Babus, the disease exhibits considerable variations in the degree of intensity of the process, all gradations between attacks of great severity and those of the mildest character being seen. In a case of this latter type the temperature never rose above 100°F. and in many cases the hæmoglobinuria has lasted only twelve hours or less.

The two cases among European children of which we have particulars were both extremely mild (*vide* Cases XIII and 67), the hæmoglobinuria being of the most transient nature and accompanied by very few symptoms.

Relapses coming on within from one to ten days or more are very common. They are often of equal duration with the first attack, but generally milder in character. They appear to follow chiefly in the more severe cases.

Temperature.—With the onset of the rigor that ushers in the Black-water Fever there is a rise of temperature to 105° or thereabouts. Taken as a whole the curve for an attack usually extends over about forty-eight hours, falling more or less gradually towards normal.

But the temperature does not necessarily cease with the hæmoglobinuria ; very commonly there is even severe and continued fever lasting many days after the hæmoglobinuria is over, a condition termed by Daniels (24) " post-hæmoglobinuric " fever. This has been noticeable in some degree in most of our cases. It is doubtful if it is malarial in nature, as it usually does not yield to quinine nor are parasites usually to be found. In mild cases the temperature does not rise above 100° or 102°, as in one case recorded by us : Case VI. Afebrile temperatures have also sometimes been recorded.

When a relapse occurs the temperature again rises and the history of the rst attack is repeated.

Splenic enlargement.—In all our cases there has been splenic enlargement. In some cases there was a history of this condition being present before the attack, and in two the spleen reached nearly to the umbilicus before the onset of the Black-water Fever. In one case this organ was seen to enlarge very markedly during the paroxysm; and in several others we have seen a reduction in the size of the spleen take place (*vide* cases) as convalescence set in. In none of our cases has splenic enlargement been absent.

Epigastric pain.—This very characteristic symptom is practically always present in greater or less degree. Pain is complained of not over the spleen and liver, but over the pit of the stomach and behind the sternum. If there is a return of the hæmoglobinuria, there is generally a return of the pain. The renal pain mentioned by some authors has not been remarked by us.

Increased pulse tension.—There is with the onset of hæmoglobinuria, an almost invariable increase in the pulse tension, which lessens as the paroxysm abates. With a recurrence of the hæmoglobinuria the high tension pulse returns.

Icterus.—Icterus is rarely present until a certain time has elapsed after the onset of the hæmoglobinuria. In some cases it may be confined to a slight yellow tint of the conjunctivæ. It is associated with the existence of an intense yellow colouring of the serum; but not with the presence of bile salts in the urine. The icterus of Black-water Fever is generally very transient, lasting only a few days after the cessation of the blood destruction; but sometimes a slight bronzing of the skin may remain for long period.

II.—Urine changes.

Hæmoglobinuria.—The first urine passed may be light red or quite dark. In the latter case it often shows on the removal of the hæmoglobin by acidifying and boiling a considerable amount of dark brownish pigment. The same brownish yellow colour is sometimes seen in the urine passed at the height of the attack. Later on, clearing by acidifying and boiling shows that this is not present, the urine resembling ordinary high coloured febrile urine.

When fresh urine passed early in the disease is examined by the spectroscope, the bands of oxy-hæmoglobin are well marked, and can easily be reduced and reinstated by shaking; but the band in the red indicative of met-hæmoglobin is often very faint, and only becomes pronounced after the urine has remained standing for some time. The condition in Black-water Fever seems therefore to

be essentially an oxy-hæmoglobinuria, the formation of met-hæmoglobin being to a large degree a secondary process.

The amount of urine passed varies greatly ; some cases exhibit polyuria, in others the amount of the urine is normal or diminished ; in some suppression occurs very early in the disease. In the most typical condition dark urine is passed at short intervals during the first twenty-four hours, the total amount being in excess of the normal.

The amount of hæmoglobin passed, calculated in terms of blood necessary to produce the colouring matter, is less than would at first sight be expected.

An estimation of the amount of hæmoglobin passed in two typical cases showing as follows :—

Case XX.	Quantity.	Percentage Hæmoglobin in terms of blood.	Amount of blood represented. c c.
		Per cent.	
Urine passed prior to—			
11 A.M., 16th January 1908	60	10·	6·
11 P.M., „	750	6·6	50·
9 A.M., 17th January 1908	550	6·6	37·
7 P.M., „	550	6·6	37·
12 P.M., „	350	6·6	23·
12 A.M., 18th January 1908	450	5·	22·5
12-50 P.M. „	300	2·5	8·
Total quantity of blood represented . . .	...	...	183·5 c.c.

Case XXI.	Quantity.	Percentage Hæmoglobin in terms of blood.	Amount of blood represented.
		Per cent.	
Urine passed to—			
4 A.M., 22nd February 1908	310	2	62·
7 A.M.—8-30 A.M.	630	3	18·9
10 A.M.	280	5	14·2
1 P.M.—2 P.M.	570	5	28·5

Case XXI.	Quantity.	Percentage Hæmoglobin in terms of blood.	Amount of blood represented.
		Per cent.	
3 P.M.—4-30 P.M.	510	3	15·3
6 P.M.	280	3	8·4
7 P.M.—9-30 P.M.	650	3	19·5
10-30—6 A.M., 23rd February 1908 . .	510	3	15·3
7 A.M.	310	2	6·2
9 A.M.	300	5	1·5
Total quantity of blood represented . .	...	...	133·8 c.c.

As in an ordinary case the red cells are reduced to at least half their normal number the amount of hæmoglobin passed in the urine can represent but a portion of the full destruction. Yet if this amount of approximately 200 c.c. of blood were dissolved in the total plasma it should make a solution of at least 10 per cent. This we shall see is much greater than was the hæmoglobinæmia actually present in any of our cases at any given time. Such a want of relation most probably indicates that excretion is more or less keeping pace with the liberation of free hæmoglobin, and may explain observations wherein the hæmoglobinæmia has not been noted.

Urobilinuria.—Throughout the attack urobilinuria is present. In estimating approximately the amount of this substance we employed dilution of the zincurobilin as produced in the qualitative test, until the bands could no longer be determined with certainty. In cases in which the quantities were estimated after boiling the acidulated urine to remove the hæmoglobin, the amount of urobilin seemed as great in quantity in the first samples passed as later when hæmoglobinuria had ceased, the bands being easily visible on twenty-fold, but not on further dilution.

For many days after the hæmoglobinuria has ceased, although hæmoglobin may not be demonstrable in the serum, urobilinuria continues. It does not therefore seem to be dependent upon the actual presence of hæmoglobin in the plasma.

In several cases of fever (malarial) where it was doubtful whether there had been hæmoglobinuria, we were able to detect the presence of urobilin in the urine in quantity as large as that found in Black-water Fever. We do not know enough to state what relation to the Black-water Fever process this conditi may bear.

Albuminuria.—Albumen is of course present in large amounts when the urine contains hæmoglobin. A considerable quantity is also present for a certain time after the excretion of hæmoglobinuria has ceased. Some interest attaches to the question of whether the passage of albumen is limited to that associated with the hæmoglobin or passed as a result of kidney changes. Our observations in this respect are limited, but the following example (Case XX) shows a remarkable proportion between the percentage of hæmoglobin and the amount of albumen as estimated by Esbach's process :—

Cases XX.	Percentage hæmoglobin in terms of blood (normal).	Albumen in grms. per litre (Esbach).	Specific gravity.
	Per cent.		
First urine passed 16th January 1908. . .	10·	1·05	1018
Urine up to 11 P.M.	6·6	·6	1018
Urine up to 9 A.M., 17th January 1908 . .	6·6	·5	1017
„ „ 7 P.M.	6·6	·7	1018
„ „ 12 P.M.	6·6	·7	1020
„ „ 12 A.M., 18th January 1908 . .	·5	·5	1018
„ passed at 12-50.	2·5	·3	1020

Sediment.—The sediment of the hæmoglobinous urine is of some significance. There is usually a characteristic flocculent deposit becoming more abundant as the hæmoglobinuria continues and giving place eventually even before this ceases to a heavy deposit of febrile urates. Microscopical examination of the first urine shows among amorphous debris an occasional granular cell, and these later increase in number. Granular casts of a characteristic nature (so called hæmoglobin casts) are present; but in many cases at the height of the hæmoglobinuria the urine deposit contains mainly isolated degenerated granular cells, without doubt derived from the kidney.

Hæmosozic value of the urine.—If the urine were hypotonic, any hæmorrhage into the urinary passages would give rise to a condition resembling hæmoglobinuria; and hæmorrhage into the kidney has been advanced as explanatory of the hæmoglobinuria of Black-water Fever [Pellerin (49)]. We have therefore in several cases estimated the hæmosozic value of the urine, the result in terms of sodium chloride being as follows :—

	Per cent.
Case XIX—	
First urine passed	1·25
Urine toward end of attack	·83
Case XXVII—	
First urine passed	1·28

The hæmosozic value of the urine therefore even when hæmoglobinuria is present, is far above that at which red blood corpuscles are hæmolysed, and the urine could not on account of its osmotic effect dissolve red cells.

Agglutinating and globulicidal effect of urine.—In Cases XXVI and XXVII the effect of the urine upon the patient's corpuscles and upon normal corpuscles was tried ; but no agglutination or recognisable hæmolytic effect was produced. The addition of normal serum (complement) to the mixture of urine and red cells led to no further result.

Presence of preservative bodies in the urine.—A remarkable feature of the urine of Black-water Fever, is that it often shows little or no tendency to putrefaction and can be kept with but little change for a long period.

III.—Blood changes.

Recorded observations upon the blood changes in Black-water Fever are very meagre and do not throw much light upon the nature of the process.

The relation that hæmoglobinæmia bears to the disease is still uncertain, and recorded observations upon the changes in the red cells and leucocytes are vague and couched in very general terms, most observers having confined themselves to noting the presence or absence of malarial parasites and pigment, estimating the loss of hæmoglobin and red cells, and recording the numerical changes occurring among the white blood corpuscles, both relative and absolute.

Especially are observations made at a very early stage, and those relating to different phases of the attack needed. For it is obvious that observations upon blood taken after the cessation of the hæmoglobinuria, although interesting as regards the evidence of the occurrence of previous blood destruction, are unlikely to give any indication of the process; the same applies to cases seen so late that one cannot be certain that the elimination of hæmoglobin by the kidneys may not be the only active process.

Paying special attention to these points we have been able in several of our cases to obtain blood, both peripheral and splenic, within a few hours of the onset, and to follow out the changes from day to day.

Reduction in the number of red blood corpuscles.—There are only two possible sources of hæmoglobin in the body, namely, the red cells of the blood and the muscles. The very obvious reduction in the number of the former in cases of Black-water Fever enables us to say that the condition is due to the destruction of the red blood corpuscles. This destruction is very marked and may proceed until the number is less than 1,000,000 per c.m.

The reduction is quite obvious to the naked eye and in severe cases is sufficiently well marked to introduce difficulties in the making of good blood films.

Hæmoglobinæmia.—In paroxysmal hæmoglobinuria the serum is generally stated to be of a red colour. In Black-water Fever there is some doubt as to whether this is necessarily the case; and one of us with Dr. Stephens has recorded cases where hæmoglobinæmia was not evident. The examination for hæmoglobinæmia is generally made by allowing the blood to clot and then examining the serum. But this method is open to grave fallacy; for we have not infrequently seen rosy serum exuded from the clotted blood, when by placing the same blood in 5 per cent. citrate of sodium solution and centrifuging, hæmoglobinæmia was not to be detected. The red colour of the serum was due in these cases then not to hæmoglobin present in the plasma during life but to changes which have taken place after withdrawal from the body. (*Vide* extra-vascular lysæmia, Part III, Chapter III.) Examination of the blood in Black-water Fever has shown without exception the presence of true hæmoglobinæmia, demonstrated by the centrifuging of blood received into hypertonic citrate solution; the serum after clotting has also always shown hæmoglobin provided the hæmoglobinuria was still in progress, but in both cases the amount was usually small and more or less masked by the extraordinarily intense yellow colouration of the serum. We have only once (Case XXVII) seen hæmoglobin so great in amount as to give a rosy colour to the serum and its presence is generally only indicated by an orange tint.

Owing to this fact it is difficult to estimate with accuracy the amount of hæmoglobin present; but in most cases seen by us it does not seem to have been more than the equivalent of a 1 per cent. solution of normal blood.

In the one case in which the serum was rosy red the percentage of hæmoglobin was as high as a 3·75 per cent. solution of blood.

The amount of hæmoglobinæmia in the serum exuded from the clot has not in our cases exceeded the amount shown by centrifuging the citrated blood. In none of our cases have we seen solution of the clot as described by Hayem in a case of Paroxysmal Hæmoglobinuria; it was on the contrary very noticeable in all our cases that however long the clot was left in contact with the serum short of putrefactive changes, no solution of the red cells took place. The amount of hæmoglobinæmia and its relation to hæmoglobinuria in our cases has been as follows:—

Case XIX (mild attack)—

(1) Ten hours after onset serum a deep yellow. A trace of hæmoglobin shown by the spectroscope. Citrated blood centrifuged shows a trace of hæmoglobin by spectroscope.

(2) Next morning hæmoglobinuria has ceased. Serum lighter yellow in colour. Presence of hæmoglobin in serum doubtful.

Case XX (severe attack)—

(1) Seven hours after first rigor serum a deep orange colour. Hæmoglobin tint can be detected with the naked eye. Citrated blood centrifuged shows a similar amount of hæmoglobinæmia.

(2) Next day, serum markedly yellow with orange tint, hæmoglobinæmia present.

(3) Day of cessation of hæmoglobinuria, hæmoglobin not demonstrable in the serum of plasma.

Case XXI (severe case)—

Six hours after onset. Serum markedly yellow with orange tint. Spectroscopically shows well marked bands of oxyhæmoglobin. Citrated blood centrifuged also shows hæmoglobinæmia marked by yellow tint.

Case XXIV (severe case)—

(1) Fourth day, hæmoglobinuria nearly over, suppression threatening. Serum shows a characteristic orange tint and bands of oxyhæmoglobin.

(2) Fifth day, serum yellow, no hæmoglobinæmia.

Case XXVI (severe case)—

Second day. Hæmoglobinuria in progress, serum rosy red. Citrated blood cenrifuged shows same degree of hæmoglobinæmia. Calculation of amount of hæmoglobinæmia by comparison of tint with laked blood, diluted to various degrees, gave 3·75 per cent. hæmoglobin.

Increased coagulability.—Though we have made no accurate estimation of the coagulation rate, we have in a number of cases noted a distinct increase in the rate of coagulation early in the disease. Later on this is less noticeable and there may be delayed coagulation. In one case it was sufficient to cause buffy coat clot.

IV.—Changes in the Red Blood Corpuscles.

Some of the earlier descriptions of the blood in Black-water Fever, remark upon the profound alterations in the shape and condition of the red cells. There can be little doubt that some of these descriptions referred to artifects; for as has since been frequently stated the general appearance of the blood in Black-water Fever is that of normal blood. The very normal appearance of the blood cells in a disease so obviously due to an acute blood destruction is one of the striking features of the pathological picture.

During the early stage of the disease it is often only after prolonged scrutiny and systematic examination of a number of microscopic fields that we can appreciate the few changes which otherwise may easily pass unnoticed.

In films taken within two hours of the onset of a fairly severe attack we failed to detect on the most careful search any abnormality whatever; nor was there a single "shadow" found in fifty microscopic fields.

Later in the attack certain changes are generally to be made out; but they are by no means so conspicuous as one might be led to expect. Thus in the blood taken about four hours after the onset of a severe attack we found, among approximately 20,000 red cells, 150 large shadow corpuscles, 129 small shadows, and 16 markedly anæmic cells. After the second or third day the blood changes are much more obvious and they often reach a maximum when polychromasia of a marked degree is established. It is these later changes, almost certainly regenerative in nature, which have been emphasized by most observers.

We shall for the sake of clearness differentiate the early changes from these later ones, which are usually even more marked in convalescence than during the continuance of the disease.

Early changes.

Size of the red cells.—In order to ascertain whether in spite of the perfectly normal appearance of the blood cells to ordinary microscopic examination, there might be some changes found on actual measurement of the cells, a series of observations were made on normal and abnormal bloods, including that of Blackwater Fever.

Films of different bloods made with as great uniformity as possible were used, and portions were chosen for observation where the red cells were distributed so as to be a little distance from one another. The cells were then passed, slowly across the field of a screw micrometer eye-piece, partially occluded by means of an Ehrlich's stop, and each cell measured carefully. This method, though rough, gave fairly uniform results which enabled one to ascertain better than by mere microscopic examination, variations in the size of the different cells.

An improvement in the method consisted in the use of a Leitz drawing eye-piece whereby accurate tracings of the outlines of corpuscles in a number of microscopic fields were made, and afterwards measured and compared.

Hayem (57) has described cells of three sizes, large, medium and small, measuring 6·5, 7·5, and 8·5 microns, respectively, of which the proportions in normal blood were large and small 12·5 per cent. each, and medium 75 per cent. In our cases it was easy to distinguish three sizes of cells, but the greater number

measured about 6·7 microns, only a few being below 6 microns and a somewhat larger number about 8 microns. At times all three measurements were proportionately increased but never diminished. Apart from polychromasia, associated always in our experience with an increase of large forms of red cell, these observations have not shown any obvious variation in the proportions of the different sizes in Black-water Fever, or in those exposed to black-water conditions, but a peculiar phenomenon we have several times observed may be here remarked upon. In comparing the drawings described above it became evident in several instances that in series of films taken at short intervals within the first twenty-four hours of the onset of Black-water Fever, all the elements of the blood, both red cells and leucocytes, were on a larger scale in those taken later on the attack than in the earlier ones. At first it was thought that some accident might account for this condition; but careful scrutiny of the films did not bear out this supposition, for our films from long practice exhibit very little variation in the conditions under which they are made, and the increased anæmia of the later stages would lead us if anything to give less pressure to the needle at the time, so that a reduction, not an enlargement of the elements, should therefore be expected. While at the present moment we do not care to place too great stress upon this phenomenon, which we intend to study more closely, we believe it indicates some definite change, either in the cellular elements or in the plasma.

Shadow corpuscles.—Shadows of red cells are frequently very indefinite and may easily escape notice. They are sometimes exceedingly large, pale, and irregular, appearing at first sight like mere empty spaces among normal corpuscles; for careful scrutiny shows these spaces to be occupied by corpuscles which have evidently become enormously swollen and softened, apparently during the process of dissolution, for they stain very faintly except at the periphery where they become moulded by the normal corpuscles surrounding them.

At other times shadows are but little larger than a normal corpuscle; but they almost always appear diffluent and are moulded by surrounding corpuscles.

In addition to these large shadows there are even more characteristic quite small fragments, markedly irregular stellate or elongated in form, evidently of very soft consistence and staining more feebly than normal red cell substance.

That these are not artifects we are convinced, for we do not find them in our films of normal or malarial blood, nor in the blood of Black-water Fever cases except at certain stages. Many of the small shadows also exhibit appearances that we do not find even in carelessly or badly made films, where of course shadows and fragments may be numerous.

Spherocytes and other abnormal conditions of the red cell seen early in the disease.—Small deeply staining round cells have been described by many authors. In pernicious anæmia and other conditions very small corpuscles may be present,

but these are not necessarily of the type to which we are now referring, so that the term microcyte would be ambiguous. These cells it is true when seen in films are smaller than the normal red cells; but this appearance is due mainly if not altogether to changes in their elasticity, which prevent them from becoming as flattened as the normal corpuscles.

As such cells, as we shall see later, are a sign of most important pathological changes, we have thought it desirable to have a name to designate the condition and have termed them spherocytes.

Though a spherocyte can be picked out from among normal cells by its small size, associated with its altered staining properties, there is no hard-and-fast line of demarcation between a normal cell and others showing in various degrees this change. Restricting ourselves to the well-marked condition we can note the following alterations as characteristic of spherocytes. (1) They are among normal corpuscles conspicuous from their regular circular outline. This is not infrequently accentuated by a circular crack produced by shrinkage of the serum from the unyielding corpuscle. (2) They not only stain more deeply, but frequently show a gradually increasing depth of colour towards the centre of the cell. (3) In fresh films they resemble the "globuli-rossi" seen in malaria and can be picked out by their darker tint and the absence of the plate-like appearance of normal corpuscles.

Among corpuscles stained by adding a few drops of blood to a saturated solution of neutral red in 3 per cent. citrate of soda solution, are some which take on a deep brown as compared with the light yellow tint of the great majority of cells. Most frequently cells with stain in this way appear to be spherocytes; but we cannot definitely state that this peculiar reaction is one confined to spherocytes.

Cells of normal size, but showing an irregular, often pear-shaped outline and pale stained centres are also seen. Cells of normal size and shape but with markedly pale staining centres are also occasionally observed.

The most distinct feature of the blood in Black-water Fever is the occurrence of the shadows we have referred to.

Spherocytes are by no means conspicuous in the peripheral blood, though as we shall see they may form a very important feature in the blood from the spleen.

Late changes.

Polychromasia and allied conditions.—A peculiarity of red cells which under suitable conditions of stain causes them to take on a bluish or slate colour, instead of the ordinary eosin-red, has been termed polychromasia. Maragliano

(73), Castellino (73), and Ehrlich (73) have considered the condition a sign of degeneration. But Engel (73) and others look upon these cells not as degeneration forms, but as a stage in the formation of the ordinary red cell; and according to Engel polychromatic cells appear when very rapid formation of red cells is demanded.

We have every reason to believe that the polychromasia seen in Black-water Fever is quite unconnected with degeneration and is due to regenerative changes.

Cells showing polychromasia in its most marked form are of very large size, measuring up to 10 microns or more, and taking on with most Romanowski's stains a more or less pale bluish purple colour. They are rarely of normal shape being more or less irregular and showing a distinct tendency towards lobulation, a feature which may be accentuated by an appearance of three or more faint lines which divide up the cell into several portions. In the most extreme cases there is a central stippling with Romanowski red, arranged in a more or less stellate manner. Though such cells may form quite an appreciable percentage of the whole, the majority of the polychromatic cells exhibit these features in a lesser degree. Associated with the polychromasia there are always a considerable proportion of more or less normal cells of a larger size than usual and others exhibiting every gradation between normal and polychromatic cells. Unless associated with the presence of spherocytes, there is therefore with presence of polychromasia a general increase in the size of the red cells, very noticeable under the microscope at the first glance.

During the early stages of Black-water Fever, polychromasia is rarely seen; it begins to be apparent after the second or third day and reaches its maximum most often when the patient is convalescent, usually several days after the cessation of the hæmoglobinuria.

Nucleated red cells are not usually seen, if at all, until late in the disease; except that they contain a nucleus they resemble in all respects polychromatic red cells. In certain cells of this nature the usual densely staining circular nucleus is not present, but its place is taken by a pair of small densely staining nuclear masses, one invariably larger than the other, but never measuring more than about 2 microns. These nuclear masses are always associated with the reddish stippling of the cells before alluded to.

So called basophilic degeneration has been recorded in Black-water Fever. In our experience it is a late and somewhat rare condition generally associated with polychromasia; and we doubt if it has any relation to susceptibility to destruction of the red cells, a suggestion sometimes made.

The extent of these changes will best be gathered from the following case.

But the discussion of the significance of the condition is best postponed until we have studied our subject on a basis of experiment—

Case XX.—Hæmoglobinuria continuing for three days. A case in which we were able to examine the blood early on the first day of the attack and regularly on the succeeding days until the patient was convalescent. Examination of the blood on the first, second, and third days, respectively, showed among 20,000 red cells counted on each occasion the abnormalities noted below—

	First day.	Second day.	Third day.
Large shadows	15	51	02
Small shadows	20	35	27
Anæmic cells	35	35	15
Deformed cells of normal size	105	48	84

In this instance it will be noticed that the blood changes were not by any means marked, although at the time when the blood films were obtained hæmoglobinæmia was present.

On the third day after onset polychromasia began to be conspicuous and large cells measuring 8 microns were as numerous as medium sized cells. On the sixth day there was an extraordinary degree of polychromasia, large polychromatic cells forming a considerable proportion of the total. Basophiles were also present.

A comparative count gave—

	Per cent.
Markedly polychromatic cells	18
Large sized corpuscles „	23
Medium „ „	53
Small „ „	4

Case XXI.—(1) In films on the first day, six hours after the onset of hæmoglobinuria, the following were found, among 20,000 red cells:—

Large shadows	156
Small shadows	33
Anæmic	16
Deformed cells of normal size	129

(2) A second film taken on the same day but several hours later showed no indication of blood destruction, for in an examination of 120 microscopic fields (approximately 20,000 red cells) we were unable to find either shadows, microcytes or anæmic cells.

(3) A third specimen of blood obtained after a further sixteen hours with hæmoglobinuria still continuing showed among 20,000 red cells—

Large shadows	12
Small shadows	6
Anæmic cells	6
Deformed cells of normal size	21

It may be noted that during the interval between the taking of the second and third specimens a slight rigor had occurred.

In this case it is of special interest to note that at no time during the passage of hæmoglobinuria in this primary attack, did the blood show the presence of polychromasia, granular degeneration of the red cells, or nucleated red corpuscles. But five days later a relapse took place, and at this time polychromatic red cells were present in large numbers, together with numerous nucleated red corpuscles of various sizes and a marked general poikilocytosis of the erythrocytic elements.

Case XXIV.—(1) Examination of films taken some hours after onset shows nothing abnormal. No shadows or spherocytes or other observable change.

(2) Films taken on the third day, while hæmoglobinuria was still in active progress show:—

	Per cent.
Large shadows	·4
Small shadows	1·1
Sperocytes	·3
Microcytes	·05

(3) Films taken on the fourth day after cessation of the hæmoglobinuria showed no shadows.

These observations are in accord with those described by Grocco (45) in paroxysmal hæmoglobinuria, in which he found little or no evidence of blood destruction on microscopical examination, although marked hæmoglobinæmia was present in the plasma at the time.

V.—Changes in the leucocytes.

Specimens of the blood taken very early after the commencement of an attack of Black-water Fever generally exhibit a relative large mononuclear leucocytosis, consisting chiefly of the ordinary typical large cells with kidney shaped, or incurved, and somewhat eccentrically placed nuclei, an increase of which has been so often noted as occurring during the course of a malarial attack. Sometimes, as has been remarked elsewhere, certain of these cells may be found containing malarial pigment. A little later in the attack certain large mononucleated cells of a different type appear in the general circulation; and it is coincident with the appearance of these new elements that phagocytosis of red cells and shadow corpuscles may be observed.

At a still later stage enormous irregular endothelial plaques appear, giving rise to the impression that a more or less extensive desquamation of the endothelium has taken place. With the cessation of hæmoglobinuria and the appearance of polychromasia and nucleated red cells there is a more or less rapid disappearance of these abnormal types of cell from the blood, though the ordinary

large mononuclear leucocytes may still be found in excess. At this stage also true lymphocytes and eosinophiles which have been reduced almost to the vanishing point during the paroxysm of hæmoglobinuria once more appear, sometimes in increased numbers. Many of the polymorphonuclear neutrophile elements which have possibly shown diminished granular staining of their protoplasm during the attack, now appear more densely granulated, a condition shared by a large number of the transitional and mononuclear elements, many of which latter approach the myelocyte type. Some of these also show a distinct tendency to eosinophile granulation; and should perhaps be described as eosinophile myelocytes.

The most striking of these phenomena is undoubtedly the appearance of the large mononuclear cells and macrophages. The disappearance or great reduction of certain of the leucocytes, and the appearance of transitional forms and myelocytes, also requires some further mention.

Increase in the large mononuclear elements.—This relative increase which is also seen to a marked degree in malaria has been sometimes considered a sign of protozoal infection; but it has been described by Hayem in a case of methæmoglobinuria of chemico-toxic origin, and it appears to us possible that it may in some way be related to the occurrence of red cell destruction.

Poch (60) has shown that it occurs in a most marked degree in benign tertian and quartan infections, being less marked in malignant tertian malaria.

In Black-water Fever the increase is always distinct though not necessarily very great. The following tabular statement gives the result of counts in Black-water Fever cases. Most of these counts include, among the large mononuclear leucocytes, cells of the macrophage type:—

Case.	Day of the disease.	Polymorpho-nuclears.	Large mono-nuclears.	Small mono-nuclears.	Eosinophiles.
Case No. 1 . .	3	57·	24·9	16·6	1·5
„ 4 . .	1	59·	20·5	20·	·5
	2	54·3	27·4	18·3	...
	3	55·6	21·6	22·8	...
	4	53·6	16·6	24·	5·6
„ 5 . .	1	49·7	26·3	23·8	·2
„ 7 . .	3	53·8	28·5	17·2	·25
„ 9 . .	1	66·3	27·8	4·7	1·2
„ 10 . .	2	48·4	26·6	24·	1·

Case.	Day of the disease.	Polymorpho-nuclears.	Large mono-nuclears.	Small mono-nuclears.	Eosinophiles.
Case No. 11 . .	2	61·	33·	6·	0·
„ 12 . .	3	49·	24·6	21·	5·4
„ 14 . .	3	59·4	26·4	8·9	5·6
„ 15 . .	4	54·2	24·8	20·8	·2
„ 16 . .	2	55·9	14·9	21·1	·4
„ 17 . .	2	48·9	20·1 10·7	20·1	·2
	4	62·3	22·3	12·7	2·4
„ 18 . .	3	65·	18·	...	...
„ 19 . .	1	83·	9·6 1·3	6·	0·
	2	67·5	8·	18·	6·5
„ 20 . .	1	63·7	23·	9·3	1·82
	2	61·7	28·7	9·5	2·1
	3	57·4	22·	14·	1·65
	4	49·4	25·5	19·8	3·
	7	73·	9·	9·	5·3
„ 21 . .	1	73·6	18· 2·2	6·	·2
	2	62·	10·2 7·2	20·5	·0
„ 24 . .	4	75·5	11·7	12·3	·3
„ 25 . .	3	56·6	13·3 5·5	23·3	·5

Macrophages.—These cells which usually appear within the first twenty-four hours after the onset of an attack of Black-water Fever are of several types—

Type (*1*).—Cells resembling the ordinary large mononuclear leucocyte, but ranging from 15 to 25 microns in diameter.

They possess a large oval, indented, or kidney-shaped nucleus placed usually a little eccentrically and surrounded by a large amount of hyaline or faintly granular protoplasm. In the larger of these cells the nucleus may measure 15 microns in the long diameter. These cells sometimes contain malarial pigment, and they may also be found occasionally with engulfed red cell shadows.

Sometimes the nucleus of these cells has divided, forming a pair of round twin nuclei.

Such cells whose appearance is that of very large specimens of ordinary mononuclear leucocytes form a marked feature in the blood of Black-water Fevers.

Type (2).—Large irregularly circular cells with a compact roundish or rhomboidal nucleus situated more or less centrally in a mass of pale staining protoplasm.

They vary in size from 15 to 20 microns or more, while their nuclei generally measure 10 to 12 microns in diameter. The larger cells of this type are frequently vacuolated while many of them contain the debris of what seem to be red cells along with distinct red cell shadows. The smaller cells of this type are evidently markedly phagocytic and may sometimes be found containing engulfed red blood cells, but little altered in appearance.

Such cells resemble except in size the small mononuclear leucocytes with hyaline protoplasm.

Type (3).—Large cells with irregular or polygonal nuclei, which lie either to one side or stretch right across the cell body.

They may attain enormous dimensions, often measuring from 20 to 30 microns in diameter. They also possess phagocytic properties frequently being found with engulfed and altered red cells, and what appears to be red cell debris.

Endothelial plaques.—The macrophages described above which appear comparatively early, usually within the first twenty-four hours of Black-water Fever and are still to be found in diminishing numbers after the actual hæmoglobinuria has ceased, appear to be free cells which have for some reason appeared in the peripheral circulation. But there are certain cells which seem to be desquamated endothelial plaques. Many of these cells are of very large size, and plaques composed of two or more joined together side by side are not uncommonly seen.

The nucleus of these cells is large and frequently stretches nearly across the cell and exhibits considerable irregularity of outline. The protoplasm which is hyaline in nature often shows extensive vacuolation and may contain red cells, debris, and shadows. Such cells resemble those found in the splenic and hepatic veins *post mortem* and endothelium cells of the visceral capillaries.

Their presence in the peripheral circulation appears to point to the action of some irritant (possibly free hæmoglobin) upon endothelium as a result of which a more or less extensive shedding of its elements takes place.

Reduction in number of certain forms of leucocytes.—Coincident with the increase in large mononuclear elements, which is most marked during a fall in the

patient's temperature, but is usually persistent throughout the attack, there is generally a decrease in the polymorphonuclear leucocytes. A most interesting phenomenon, which we find to hold good in a number of cases, is the great reduction of true lymphocytes, as opposed to small mononuclears, and the frequent partial or total disappearance of the eosinophile cells during the early stages of the disease followed by the reappearance of these elements, sometimes in considerable numbers at a latter stage.

Transitional cells and myelocytes.—Towards the end of the attack a large proportion of the polymorphonuclear cells contain nuclei much less lobulated than normal, and such cells appear to be immature forms of polymorphonuclear leucocytes. Other cells of similar nature are of more or less purely mononuclear type, and as we have noted, appear to be neutrophile myelocytes. Their presence at this late stage of the disease seems to indicate an attempt on the part of the blood forming organs to repair a loss occasioned by an extensive destruction of the polymorphonuclear forms during the earlier stages of the malady.

Red cell phagocytosis in the peripheral blood.—Although red cell phagocytosis may not be conspicuous on casual examination of the blood of Black-water Fever cases, some evidence of its occurrence can invariably be found on careful search especially during the earlier stages of the disease.

Such evidence is generally in the form of large clear engulfed stromata of red cells which are more or less devoid of hæmoglobin. But in a few instances macrophages may be found containing but little altered red cells. Later in the disease when endothelial plaques have made their appearance, a large proportion of these elements may be seen containing one or more engulfed and decolourised red corpuscles.

Extrusion of nuclear matter in certain types of cell.—Before leaving the question of the blood changes observed in Black-water Fever we must record a peculiar feature of the small mononuclear leucocytes observed in one case only in blood films taken on the fourth day of the disease.

In these specimens a large proportion of the small mononuclear leucocytes which possessed a considerable amount of protoplasm exhibited a deeply staining mass of nuclear like substance lying for the most part just within the protoplasm at the periphery of the cell. In some instances there was an indication of some fine protoplasmic threads extending from this body towards the cell nucleus, and in one it appeared as though the body might have been just extruded from the cell. In general these masses were quite minute, measuring no more than one to two microns in diameter, though a few which appeared to be of a more elongated shape were slightly longer. Judging from their appearance and staining reactions it is probable that they are of the nature of nuclear extrusions associated possibly with cell proliferation. The slightly indented form of the

nucleus of the leucocytes containing them seems to give some warrant for this idea.

VI.—The examination of splenic blood.

In a previous paper we have recorded the result of an examination of the splenic blood taken at an early stage in the course of an attack of Black-water Fever. The most prominent feature in this case was the presence of phagocytosis of red blood corpuscles, many of them quite normal in appearance, both as regards the absence of parasites and the possession of their full hæmoglobin value. Since this observation we have had further opportunities of studying the splenic blood in Black-water Fever. The examination of splenic blood in regard to the existence of a special parasite and evidence of malarial infection we have already recorded; in our present connection it is the changes likely to throw light on the nature of the mechanism of Black-water Fever, which concern us.

The red cells.—Unfortunately puncture of the spleen in Black-water Fever, except in one case, has given us only sufficient blood for the preparation of films. In Case XXVI, however, sufficient blood was obtained to enable us to stain a portion by the neutral red method already described. In this case the characteristic small dark cells were far more numerous than we have seen under any other condition, but they still formed only a small proportion of the whole.

Spherocytes have been a marked feature in almost every case, reaching a proportion to the total cells much greater than in the peripheral blood.

In one case at least there was a distinct tendency for the red cells to be gathered into groups (agglutination), such as is seen in the case of dogs injected with specific hæmolysin (Part III, Chapter II).

Splenic cells.—Many distinct types of splenic cells are distinguishable, and it only appears necessary to refer to those that seem concerned in the Black-water Fever process—

Type(1).—Large cells with a nucleus somewhat resembling that of the large mononuclear leucocytes, but considerably larger, and with more or less voluminous finely granular protoplasm. Two varieties of these cells are seen; those whose protoplasm is compact like that of the ordinary mononuclear leucocytes, and others in which the protoplasm is full of vacuoles resembling bubbles of all sizes.

But the compact and vacuolated forms have similar nuclei and may not infrequently be seen as neighbour cells in a detached fragment of endothelium. If our conception of their nature be correct, they are the product of the fixed visceral endothelium. They are extremely active phagocytes, and actively engage themselves in the engulfment of red cells, and more especially of red cell shadows. Cells resembling these but usually of the smaller type are seen

in the peripheral blood ; they are also very abundant in the capillaries of the liver, where they are seen laden with malaria and blood derived pigment.

Type (2).—Cells with irregular or polygonal nuclei similar to type (3) described as occurring in the peripheral blood.

Type (3).—Very large cells with round nuclei and voluminous more or less granular and homogenous protoplasm, often showing many inclusions. They often contain masses of malarial pigment and are not seen in the peripheral blood.

Phagocytic endothelial cells.—Quite distinct from the large apparently "fixed" macrophages are cells which take a much more active part in the phagocytosis of red cells. Prior to the inclusion of red cells, they are seen as quite small cells somewhat resembling lymphocytes. They constantly show a projection of their somewhat scanty protoplasm indicating that they are actively amœboid in nature. Their nuclei and protoplasm also show certain differences which enables one easily to distinguish them from lymphocytes. Many of these small cells are seen to contain a single red blood corpuscle, a body which would seen almost too large for them to ingest. With their included red cell, they appear of circular outline ; their protoplasm being stretched in a narrow arch round the engulfed erythrocyte, whilst the nucleus from the pressure of this same body had become crescentic.

In spite of the apparent discrepancy between the amount of their protoplasm and the size of the bodies they include, these cells ingest sometimes two, three, or more red corpuscles and all stages are seen up to cells that have engulfed a dozen or more of these. Even in the latter case, the nucleus is still quite small ; and the protoplasm is only just sufficient to retain the engulfed corpuscles. The exact nature of these cells is not clear ; but they appear to be concerned especially in red cell phagocytosis. They are rarely seen in the peripheral blood.

Phagocytosis of red cells.—Phagocytosis of red cells has been described in a number of diseases. A certain degree of this condition has been found even in health, especially in old age. In several spleens of more or less pathological interest and in the spleen of animals we have found a certain amount of red cell phagocytosis, but in proportion negligible, when compared to the extent of the condition present in Black-water Fever.

Several distinct conditions are to be seen—

(*a*) Engulfed cells more or less normal in appearance.

(*b*) Large, clear, circular spaces, that have been occupied by red cell stromata, now completely decolourised.

(*c*) Numerous small fragments of red cell substance, and small vacuoles which appear to owe their derivation to previously ingested red cells.

The included red cells in these circumstances are either quite normal in appearance, or partially decolourised, but rarely shrunken or deformed. Many such cells are evidently spherocytes. In the case of the phagocytic endothelial macrophages, cells containing hæmoglobin seem to be engulfed, and to undergo decolourisation within the ingesting cell. If, for example, a phagocyte of this type is seen to contain several red cells, one may be unaltered, while the others show all gradations between this and complete decolourisation.

But the cells of large mononuclear type and mononuclear leucocytes, though found with apparently unaltered cells, are most frequently seen to contain a number of the decolourised stromata described. The appearance presented by these cells is therefore in considerable contrast to that of the larger phagocytes, whose protoplasm is stretched by unaltered and partially altered red cells.

The actual observations, so far made, have been as follows :—

Case XIX.—Extremely mild. Spleen punctured shortly before the hæmoglobinuria ceased, and about 8 to 10 hours after the onset—

	Per cent.
Large shadow corpuscles	·05
Small poikilocyte shadows	·2
Spherocytes	·15

No tendency to agglutination of red cells observed.

Engulfment of red cells and stromata fairly conspicuous, included cells being nearly all very small spherocytes.

Case XX.—A severe case. Spleen punctured about nine hours after first rigor and about 48 hours before cessation of hæmoglobinuria—

Large shadow corpuscles Small poikilocyte shadows	Not at all conspicuous
Spherocytes	·7 per cent.

Tendency to agglutination not observed.

Engulfment of unaltered red cells by phagocytes extremely noticeable (*vide* case in Appendix). The small type phagocite containing a single red cell very conspicuous. Shadow inclusions less conspicuous though numerous.

Case XXII.—A severe case. Spleen punctured about 30 hours after onset of the hæmoglobinuria which was still in progress—

	Per cent.
Large shadow corpuscles	·15
Small poikilocyte shadows	·15
Spherocytes	·1
Large pale cells apparently decolourised and not resembling polychromatic cells	·7

Agglutination not noticeable.

Immense macrophages, with included red cells and engulfed small mononuclear elements. Macrophages contain a considerable amount of malarial pigment (malignant tertian) including segmenting reliquats, but red cell inclusion not very noticeable. Vacuolated macrophages of mononuclear type contain numbers of colourless red cell stromata.

Case XXIV.—Case seen on fourth day. Hæmoglobinuria continuing, but showing signs of clearing up.

Splenic blood examined.

Shadows very numerous, but film having been made from very small amount of blood artifects cannot be excluded.

Spherocytes . . . . . 6·6 per cent.

Agglutination of red cells very distinct, the cells being in clusters such as those observed in dogs injected with agglutinating serum.

Blood more profoundly altered than in any case yet seen. Recent severe malignant tertian infection evidenced by the abundance of segmentation reliquats (pigment blocks) and by the finding of a segmenting malignant tertian parasite.

Case XXVI.—A severe case seen on second day. Spleen punctured about 36 hours before hæmoglobinuria ceased.

Large shadow corpuscles . . . . . ·1 per cent.
Small poikilocyte shadows . . . . . ·05 „
Spherocytes . . . . . ·4 „

Agglutination marked.

Red cell phagocytosis conspicuous. Engulfed colourless stromata in large vacuolated macrophage of mononuclear type very noticeable.

These cases serve to show that in the spleen there is not any greater evidence of blood solution (cell shadows) than in the peripheral blood. The presence of agglutination and spherocytes together with the phagocytosis of red cells and stromata, as will be seen later, are of extreme significance.

Macrophage inclusions.—Since every observation relating to the conditions present in Black-water Fever may have value, we have studied the inclusions to be found in the various macrophages.

The most notable are:—

(*a*) Included red cells, colourless stromata, and malarial pigment.

(*b*) Greenish masses undoubtedly derived from red cells which have undergone shrinkage and change in staining reaction. This appearance is seen in other than Black-water Fever conditions, in which disease it is not a very conspicuous feature.

(*c*) Particles of substance staining like chromatin. Many of these resemble blood platelets, to others it is impossible to assign any exact significance. They are seen in other conditions than Black-water Fever and are not apparently of any special interest.

(*d*) Engulfed cells.

Engulfment of nucleated cells.—In several of our cases this has been a very conspicuous phenomenon. The great majority of the included cells are found in the endothelial plaques, which may contain three or even four or five of these bodies.

The included cells are, in the great majority of cases, very small mononuclear cells, but occasionally what appear to be polymorphonuclear leucocytes are seen. Such included cells lie as a rule in a vacuole and the nucleus takes on the very intense stain characteristic of necrotic nuclear matter. The condition will be referred to later when discussing the examination of the liver in sections.

VII.—Examination of the Tissues.

The tissues of Black-water Fever cases have been described by a number of observers. The most obvious recorded appearances are those due to the preceding malaria; but certain changes are peculiar to the Black-water Fever process, notably an abundant deposit of yellow pigment in the liver cells, and certain changes in the kidney.

Our own experience has been limited to two fatal cases now described (Cases II and III). Both of these cases were very suitable for study in that death occurred during the actual course of the disease and while hæmoglobinuria was in progress. Unfortunately we were only able to obtain very partial autopsies so that only the spleen, liver and kidneys have been examined

Spleen.—In both cases recent malarial pigment was abundant. More important in our present connection was evidence of red cell phagocytosis. This was most marked in Case III, but was also present in Case II. In the former almost every macrophage showed included red cells, whilst smaller mononuclear cells containing one or more were frequently seen lying free in the splenic sinuses. Large cells containing many red corpuscles as well as smaller cells containing one or two of these were also very noticeable in the radicles of the splenic vein.

Liver.—The lobules showed no marked general changes. In the smaller radicles of the portal canal system especially in limphoid-like tissue were many cells loaded with malarial pigment. The lobular capillaries contained an almost continuous chain of large oval swollen cells, lying free like polypi in the lumen. In many cases in which the capillaries were seen in cross section these cells appeared nearly to occlude the lumen. The cells which were quite distinct from the capillary endothelium appeared to be attached to it at a single point. Some of these cells contained malarial pigment, others engulfed red cells. Most conspicuous were included nuclear masses staining an intense black with Heidenhain's hæmotoxylin. Many such inclusions, seen in every field of the microscope, gave a very peculiar appearance to specimens viewed under a low power.

Many of the liver cells, but not all, contained granules of a light golden yellow or dull yellow pigment. The granules lay in clusters, often in vacuolic areas, either diffused through the cell or more or less aggregated. Many of the liver cells showed fatty globules.

In the large perilobular vessels were a few macrophages ; and in the hepatic veins large cells containing many red cells, as well as others containing included nuclear masses, were not uncommon.

Both the hepatic and portal venous radicles contained a great deal of granular matter in which apparently normal red cells lay embedded.

Kidney.—The kidneys in Black-water Fever have recently been closely studied by Werner (58).

The glomeruli and the upper portions of the convulted tubules according to this author show a finely granular exudation. As the tubules are followed in their course the granules are seen to become coarser, and in Henle's tubules are seen coagulation like masses of the same substance. There is also a general distention of the tubule and in severe cases degenerative changes in the epithelium.

In both of our cases similar appearances were present.

In the glomeruli a faint pinkish eosin stained granular substance was present containing a few desquamated capsular cells. In the upper convoluted tubules a similar substance was seen with very little evidence of epithelial desquamation. As one passed downwards profound changes in the epithelium were to be seen, and the lumen was loaded with cast off cells. Also in addition to the fine granular matter were granules of a peculiar consistence, and those absent in the upper parts became larger as one approached the collecting tubules.

In Henle's loops the substance formed a more or less adherent mass lying around the periphery of the lumen.

In the straight ducts of Bellini the granules were nearly the size of red blood corpuscles and had somewhat the appearance of red cell substance.

In the vessels of the glomeruli and of the rest of the kidney the red cells appeared normal and there was little or no granular material in the lumen of the veins, these containing red cells of normal appearance.

A great many of the conditions we have described are evidently but the necessary results of blood cell destruction.

Thus hæmoglobinæmia of a certain degree is responsible for the hæmoglobinuria and for the icteric tinge. Other changes may, or may not, be necessary consequences of blood destruction.

It is evident that before we can make use of the data outlined, it is necessary to study the process of blood destruction or hæmolysis, and especially the conditions other than Black-water Fever in which hæmoglobinuria occurs.

CHAPTER II.

THE RELATION OF BLACK-WATER FEVER TO OTHER HÆMOGLOBINURIAS.

I.—PARASITIC HÆMOGLOBINURIAS.

In the ox, dog, horse, and sheep hæmoglobinurias due to piroplasmosis are well known. Infection by piroplasma may however occur in an acute or a chronic form; it is in the former only that hæmoglobinuria occurs.

Cattle introduced into a Red-water country rapidly contract the acute disease. The animal is intensely ill, its temperature is raised, and it passes hæmoglobinous urine. In the case of death the tissues are pale and tinged with yellow, the blood watery, and the spleen greatly enlarged. In Red-water countries hæmoglobinuria in cattle is often associated with an attack of Rinderpest; the explanation usually advanced being that the Rinderpest, by lowering the resistance of the animal, allows the latent piroplasma infection to assert itself.

Piroplasma infection in the dog is very frequently associated with hæmoglobinuria, especially in the case of susceptible animals. Dogs newly introduced into a country where piroplasmosis is endemic are very apt to contract the disease a very short time after arrival. We know of thirty hunting dogs brought out from England to Madras, all of whom within three weeks of their arrival in India were found to be suffering from piroplasmosis; a few weeks later they had all either died or been destroyed as useless. Native dogs suffer less often from hæmoglobinuria though the presence of parasites in the blood especially among puppies is very common.

As in cattle a susceptible dog attacked by piroplasmosis is severely ill. If it gets over the initial disease it may die after a long period of chronic infection, and many of those which eventually survive do not recover at all quickly but remain for a long time emaciated and intensely feeble. In our experience if a dog has passed the first acute period of the disease he may die but he will not suffer from hæmoglobinuria.

The hæmoglobinuria is always associated with an immense number of parasites which, however, may become greatly reduced in number after the access; but a young dog may die from a severe infection in twenty-four hours without exhibiting hæmoglobinuria. In a dog dying of an acute infection there is marked pallor of the tissues, and in the case of hæmoglobinuria a yellow tint. The blood is watery and the spleen more or less enlarged. If hæmoglobinuria has occurred, the kidneys are maroon coloured and swollen; but this is not present in attacks, however acute, unaccompanied with hæmoglobinuria.

With the condition in the horse and sheep we are not familiar; but parasites are demonstrable in the blood in both cases without difficulty.

A resemblance of this disease to Black-water Fever is evident; but closer examination reveals an important difference in that the experience of all observers shows parasites to be easily demonstrated both in the blood and organs.

In Black-water Fever we have seen that the closest observation fails to show a piroplasma like parasite in the blood and tissues; and that this disease is not due to any direct action of the malarial parasite is shown by considerations already given. There seems then at first sight but little connection between the mechanism of parasitic hæmoglobinuria and that seen in Black-water Fever.

II.—Dyscrasic Hæmoglobinurias.

Hæmoglobinuria occurs in man in other conditions than Black-water Fever. The most important from our point of view is paroxysmal hæmoglobinuria.

Paroxysmal hæmoglobinuria was first studied by Harley in 1864 (61). Since then many observers have contributed to our knowledge of the condition, the exact nature of which is still unknown. After an exposure to cold, the patient has a violent rigor with chattering of teeth and spasmodic action of the respiratory muscles; at the same time the number and hypogastric regions become painful. The face and extremities are cyanosed. The urine at first reddish becomes later dark or blackish in colour. It is devoid of blood cells and contains both hæmoglobin and met-hæmoglobin.

After the crisis the urine becomes clearer again but is somewhat darker than normal; and contains albumen. The crisis is accompanied by a rise in temperature. Tumefaction of the spleen is frequently present; and there may be some enlargement of the liver. In severe attacks there is a subicteric tint of the integuments; but no Gmelin's reaction is to be obtained with the urine. Urobilinuria is in these cases habitual.

An ordinary attack of paroxysmal hæmoglobinuria lasts a few hours only.

An autopsy described by Hayem showed the kidneys sepia coloured, with the convoluted tubules and loops of Henle infiltrated with hæmoglobin.

We have then a disease which except in its ætiology and its comparatively mild nature is a close counterpart of Black-water Fever; and as Black-water Fever is generally induced by quinine, so this condition is precipitated by so simple a cause as exposure to cold. The serum in paroxysmal hæmoglobinuria has been observed by Ehrlich (62) and others to possess a "*rouge cerise*" colour and an "*aspect laque.*" But Bensaude (63) notes that this is not constant, while in one case the serum was more coloured in the

intervals between the attacks than during the attacks themselves. This "*rouge cerise*" colour is also seen in other affections, notably certain infectious diseases. As previously pointed out we have seen it when the blood received into citrate solution and centrifuged showed no hæmoglobinæmia to the present.

Bensaude states that the reason for the phenomenon is the solution of the clot in the serum; this we have seen does not take place in Black-water Fever.

In paroxysmal hæmoglobinuria the blood destruction is an indirect effect of the cold which has induced the attack; for blood exposed to cold does not exhibit hæmolysis. Bensaude also points out some difficulties in the way of the ordinarily accepted view of the disease. He shows that the actual red cell destruction and the hæmoglobinæmia is small and yet hæmoglobinuria results; whereas in the dog it is necessary to inject distilled water to the extent of twice the total volume of the blood before hæmoglobinæmia is intense enough to cause hæmoglobinuria. The most recent work on paroxysmal hæmoglobinuria is that of Widal and Rostaine (64), who satisfy themselves that the condition is due to a lack of protective body "antisensibilisatrice" in the blood, a condition which they were able to rectify by the prepared serum of a rabbit containing excess of this substance. The result of this treatment was that after the injection of the rabbit's serum the patient could plunge her hands into ice-cold water, without bringing on an attack; whereas previously such an act was always followed by a crisis.

Hæmoglobinuria of the same nature has been observed in connection with Raynaud's disease. Barlow (65) records a case of Raynaud's disease in which there was hæmoglobinuria, epigastric pain, and temporary splenic enlargement; and Dickenson (66) notes a case where an attack of paroxysmal hæmoglobinuria was replaced by local axphyxia of one hand. Jaundice is also recorded following local asphyxial conditions.

It is interesting to note that Barlow calls attention to the relation of "ague" to Raynaud's disease, several cases having occurred at short periods after recovery from tertian ague.

Another condition in which hæmoglobinuria occurs is after extensive burns. The earlier observations on the blood in such cases, ascribed the distortion, subdivision and granulation of the red cells present in the condition to the effects of trauma. Later observers do not find sufficient changes in the red cell to account for their death and believe toxic substances to be developed in the blood and tissues. Locke (67) shows that an immediate decrease of 1,000,000 to 2,000,000 red cells may take place in severe cases and up to 4,000,000 in fatal cases.

A dissolution of the leucocytes has also been recorded. Duodenal ulcer associated with burns has been suspected by Hunter (68) to be caused by the secretion of toxic substances in the bile as a direct result of the absorption of damaged blood and tissue cells.

Hæmoglobinuria following transfusion is a well known condition. Diseases in which hæmoglobinuria occasionally occurs are syphilis, septicæmia, yellow fever, malignant jaundice, enteric fever, scarlet fever, typhus fever, variola, scurvy, epidemic hæmoglobinuria of new born infants; and a few cases have been recorded as having been met with in association with influenza, leucocythæmia, pernicious anæmia, and Wenkel's disease. Other conditions in which it has been known to occur are sunstroke, exposure to excessive cold, and to the X-rays. A condition of considerable interest, of which unfortunately but little is known, is hæmoglobinuria of horses. This disease, which is only met with in well bred animals, appears to result largely as the result of rich diet with insufficient exercise. It occurs when horses that have been stabled for some time are exposed somewhat suddenly to fatigue. The hæmoglobinuria is associated with congestion and rigidity of the muscles, especially of those of the hind quarters. Lucet (6) believes the condition to be a kidney lesion, but there seems little ground for this view.

Poor conditioned horses fed on low diet do not develop the condition in spite of fatigue or exposure.

III.—Hæmoglobinuria due to osmotic conditions.

Hæmoglobinuria following the injection of distilled water into the blood stream has been described by Ponfick (69) and Hunter (68). Ponfick found it necessary to use water to more than twice the blood volume before hæmoglobinuria resulted; he also noted that before hæmoglobinuria was produced there must be intense hæmoglobinæmia.

Hunter records hæmoglobinuria in rabbits as the result of the intravenous injection of quantities up to 70 c. c. of distilled water; but the hæmoglobinuria appears to have been transient and its presence shown chiefly by the *guaiacum* reaction, and the production of a yellow deposit which Hunter believes to have been hæmoglobin.

In these rabbits injection of 70 c. c. of water was not followed by any visible change in the blood or by the presence of ghosts. But the spleen was in some cases dark and swollen. In the case of one rabbit a large number of red corpuscles showed bud-like projections, and similar buds were seen free and enclosed in cells.

These experiments, it must be acknowledged, scarcely reproduce the condition of a general lowering of the hæmosozic value of the plasma to such a point as to cause hæmolysis, but they serve to show the comparatively small effect of the injection of water unless in very large amount.

IV.—Toxic Hæmoglobinurias.

A large number of chemical substances destroy red blood corpuscles and many of these cause within the body intense blood destruction, often associated with hæmoglobinuria. Some substances cause simple liberation of the hæmoglobin; others have a special action on this substance converting it into methæmoglobin or other compounds. A close study of chemical and other effects of these poisons is not necessary except in so far as they are likely to throw light on the nature of blood destruction in the body and the causation of hæmoglobinuria.

Of experimental work on these lines the most instructive work known to us is that of Hunter (68). It will be necessary to consider somewhat fully his results.

Glycerine.—Hunter observed that the injection of a small dose of glycerine into the blood of rabbits is followed by hæmoglobinuria, though the destruction of corpuscles may be so slight in amount that scarcely any evidence of it is to be found in the blood or elsewhere.

Upon this and other facts he bases his conclusion that hæmoglobinuria is the result of liberation of hæmoglobin in the systemic circulation, however small the amount may be; thus differing in his conclusion from Ponfick, whose results have been alluded to.

Toluylendiamin.—Injection of large doses of this drug into rabbits is attended with great blood destruction; but rarely with hæmoglobinuria. In the cat very small doses cause intense hæmoglobinuria. In the dog it causes jaundice without hæmoglobinuria.

In rabbits the evidence of hæmolysis is confined to the spleen, except in the case of large doses when it extends to the portal blood within the liver. No evidence of blood destruction is to be seen in the peripheral blood, either during life or after death.

In the spleen the red blood corpuscles are seen to throw off buds, and many stromata and globules derived from the red cells are present. Cells packed with red corpuscles are noted in some instances. A remarkable fact demonstrated by Hunter was that excision of the spleen does away to a large extent with the poisonous effect of toluylendiamin on rabbits. Chemical investigation, however, did not show that the drug accumulated in the spleen in greater amount than elsewhere; but that on the contrary in three hours the amount in the spleen had

become too small to be estimated. From these facts Hunter comes to the conclusion that toluylendiamin acts in rabbits in some indirect manner.

Pyrogallic Acid.—When injected into rabbits pyrogallic acid leads to intense blood destruction associated with albuminuria and blocking of the renal tubules with hæmoglobin. The blood shows enormous numbers of globules derived from the red cells, and many of the corpuscles are markedly altered. The spleen is swollen, dark in colour, and contains enormous numbers of splenic cells filled with pigment. Red cell phagocytosis is also a prominent feature recorded by Celli and Marchiafava (95). The liver cells do not show the pigment seen in toluylendiamin poisoning.

The part played by the spleen in the blood destruction by pyrogallic acid is emphasized by Hunter who noted within three minutes after injection a swelling of the organ sometimes to three or four times its normal volume. Also, as early as fifteen minutes after injection, cells enclosing many red blood corpuscles, often only slightly altered in appearance, were seen in the spleen ; but not in the liver or elsewhere. The corpuscles in the peripheral blood in many cases were perfectly normal, showing no crenation, while the plasma was quite free from granules. In the spleen, on the contrary, many of the corpuscles were crenated, of dark colour and exhibited other changes. Unlike poisoning by toluylendiamin that by pyrogallic acid was not prevented by excision of the spleen. But following excision globules derived from the red blood corpuscles were more frequently seen in the peripheral blood.

These experiments show that pyrogallic acid, which directly affects the red cells, acts by causing these to be held up immediately by the spleen, and that evidence of blood destruction and alteration in the peripheral circulation is very slight.

In the case of rabbits poisoned by toluylendiamin the drug does not act directly upon the red cells ; but the toxic substances, whatever they may be, are derived apparently from body cells under the stimulation of the drug.

To the action of other poisons producing hæmoglobinuria it is necessary to refer only briefly.

Most of these substances as a result of direct action upon the blood produce met-hæmoglobinæmia. A certain number act indirectly like toluylendiamin. Others have a resemblance in their action to specific hæmolysins.

Saponin Group.—Bodies closely related to the glucosides which act upon the lipoids of the blood corpuscles. All cause hæmolysis, some in dilutions of 1 in

100,000. Some produce hæmoglobinuria; others do not. After experimental injection of such substance a latent period has been observed, lasting 24 hours or more, before the onset of symptoms of blood destruction.

Snake venom.—The snake venoms are related to the bacterial and specific blood toxins. They possess in varying degrees a hæmolytic action and in connection with viperine poisoning in particular, hæmoglobinuria may occur.

Post mortem examination shows changes varying with the venum used. In the case of daboia venom there is intense œdema and extravasation of blood at the site of the injection, the blood is dark, and the organs congested. Histologically are found, in addition to other conditions, vessels plugged with thrombi composed of more or less hæmolysed agglutinated red blood corpuscles. Lamb (72) has shown that if an initial small dose of daboia venom is given, coagulability is diminished; but that when a fatal dose is rapidly absorbed coagulation is increased.

Bacterial Hæmo-toxins.—Fischer and Adler (73) have obtained an intense anæmia in rabbits after injections of cultures of streptococci; and Grawitz (74) have observed a reduction in the red cells to 300,000 in a case of streptococcus septicæmia.

Experimental injection of filtrates or cultures of typhoid, cholera, anthrax, and megatherium may produce hæmoglobinuria. Special hæmolytic substances have been isolated from cultures of certain bacteria. Among the best known are tetanolysin, pyocyanolysin, staphylolysin and typholysin.

V.—Hæmoglobinuria following the injection of specific hæmolytic sera.

In the consideration of the toxic hæmoglobinurias we have dealt mainly with the conditions in which the blood is destroyed in a way more or less artificial and greatly different from that which is likely to be concerned in Blackwater Fever.

The nearest approach to hæmo-toxic conditions likely to arise in the course of a natural morbid process is to be obtained by the use of specific hæmolytic sera. In 1898 Belfanti and Carbone (75) showed that the serum of a horse injected with red cells of the rabbit acquires a special toxicity for rabbits; subsequently many other observers extended these observations. It is now generally recognised that whenever the blood of an animal A is injected into that of another species B, the serum of the latter becomes capable of causing profound intoxication when injected into any animal of the same species as A.

Bordet (85), Gruber (85), Ascarelli (85), Landsteiner (85) and Von Dungern (85) have specially investigated this form of toxicity together with Levaditi (76),

Celli, Cassagrandi and Carducci (77), while the reactions of the same nature, to be observed *in vitro*, have occupied a host of workers, prominent among whom are Ehrlich and Morganroth (78).

It seemed to us important, in order that we might obtain an insight into the processes concerned in Black-water Fever, to undertake certain experiments of this kind, and to ascertain, if possible, the mechanism of the hæmoglobinuria that can be produced in animals by the use of these specific hæmolytic toxins.

The experiments carried out by us have been made on dogs injected with the serum of a goat immunised by repeated injections of defibrinated dog's blood. The goat received a number of such injections repeated at intervals of a few days, which had the result of producing within a month a serum both hæmolytic *in vitro*, and markedly toxic *in vivo*.

Our experiments with this serum are as follows :—

Dog. 1. Weight 3½ lbs.

6 p.m. 10th February 1908.—Received 4 c.c. goat's serum.

8 a.m. 11th February 1908.—Dog quite well. There is no distinct pallor of tongue. Received another 2·5 c.c. serum.

8 a.m. 12th February 1908.—Tongue pallid. Conjunctivæ pale.

10 a.m. 12th February 1908.—Pallor much increased. Blood watery, dog dazed, resembling a case of acute airoplasmosis. Urine pale.

11 a.m. 12th February 1908.—Autopsy.

[Tissues pale.

[Spleen enormously enlarged. Weight 20 grammes. Firm and black in colour with a tuberculated surface.

Liver not obviously enlarged. Dark in colour.

Kidneys normal in appearance.

Urine in bladder free from hæmoglobin or urobilin.

Mediastinal glands are many of them dark red in colour.

Blood resembles a weak emulsion of corpuscles. That flowing from the hepatic vein shews to the naked eye an agglutinated appearance. Received into $\frac{N}{8}$ salt corpuscles are hæmolysed.

Hæmoglobinæmia as tested by receiving blood in 1 per cent. salt solution and centrifuging not detectable in any of the following situations :—

Peripheral blood.
Heart blood.
Blood from mesenteric vein.
Blood from hepatic vein.
Blood from inferior vena cava.

But blood separated from clot of peripheral blood was rosy red.

Microscopical examination of organs.

Spleen.—In section the organ is seen to be engorged with blood. Beneath the capsule and near the trabeculæ is a layer of fused red cell substance. In other parts the splenic sinuses are packed with more or less normal looking corpuscles. Everywhere are seen large cells gorged with red blood corpuscles.

In films are seen small phagocytes containing each a single red blood corpuscle more or less normal in appearance. Also very large cells containing a dozen or more red cells. A count made from blood obtained with a syringe from the spleen when first exposed as follows :—

	Per cent.
Polymorphonuclears and transitional cells	26
Large mononuclear leucocytes	4
Macrophages	4·7
Small mononuclear cells of various types	26
Undifferentiated cells and nuclei	34
Small phagocytes with red cells	·3
Large macrophages with red cells	2

The microscopical appearances in these films resembled extremely closely those seen in films from splenic blood in Black-water Fever.

Liver.—A count of cells seen in films omitting liver cells gave :—

	Per cent.
Polymorphonuclear leucocytes	36
Mononuclear cells and macrophages	3
Small mononuclear cells of various types	25
Undifferentiated cells and nuclei	33
Small phagocytes containing red cells	4
Large macrophages containing red cells	1

In many of the large macrophages were granules of greenish pigment resembling that seen in these cells in Black-water Fever.

Kidney.—A few phagocytes with red cells in all cases much altered.

Bone Marrow.—One altered red cell only found in a mononuclear cell after some search.

Peripheral blood.—A leucocyte count gave :—

	Per cent.
Polymorphonuclear leucocytes	74
Large mononuclear leucocytes	4·5
Macrophages	·5
Small mononuclear leucocytes	3·5
Lymphocytes	4·5
Transitional cells	12·5

But blood separated from clot of peripheral blood was rosy red.

Microscopical examination of organs.

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Lymphocytes	4·5
Transitional cells	12·5

There was marked polychromasia an average of 2 or 3 large cells with central red stained stippling (Giemsa).

Visceral blood.—In the blood from the hepatic veins are large numbers of small and large phagocytes containing red cells. Some of the latter are more or less globular with the nucleus deformed and pushed to one side by masses of included red blood corpuscles. Large scale like endothelial cells containing red cells and *débris* are common. The red cells are agglutinated in masses and between the masses are many stromata.

Dog II.—A somewhat emaciated dog. Had a large spleen resembling that found in chronic piroplasmosis but no parasites were found. No engulfment of red cells such as seen in the last case. Macrophages contained only much altered cells or *débris*.

Blood received into 5 per cent. citrate,
5 times diluted. — No tint of hæmoglobin.
Blood allowed to clot. — Serum rosy red.

Dog IV.—Weight. 4½lbs.

10 a.m., 2nd March 1908.—Received subcutaneously 15 c.c. goat's serum.
Isotonic point . . . . . ·525 salt.

1 a.m., 2nd March 1908.—Dog seedy, shivering.
Ditto — Anæmia, but no hæmoglobinuria or hæmoglobinæmia.
Ditto — Corpuscles in 1% salt at 37, no hæmolysis in 2 hours.
Ditto — Isotonic point . . . . . ·71 salt.

2 a.m., 2nd March 1908.—Isotonic point shows a trace of colour at ·83 salt. No hæmoglobinæmia.

5 a.m., 2nd March 1908.—Dog killed. Tissues pale, blood watery.

Spleen greatly enlarged, firm, black in colour, surface tuberculated.

A trace of hæmoglobinæmia in the peripheral blood and in the blood from the splenic and hepatic veins.

Urine in bladder not hæmoglobinous.

Hæmosozis value very high, being over 1·2 per cent. salt.

In this dog hæmoglobinæmia appeared to have only just supervened. It was already present in all situations. The urine was not yet hæmoglobinous.

Dog V.—Weight 5 lbs.

10 A.M., 2nd March 1908.—Received 3 c.c. goat's serum intravenously.
Dog dead in 30 minutes.

Tissues pallid, spleen enlarged, dark purple, soft and not tuberculated as in dogs 1 and 4.

Liver dark purple.

Intestine purplish. In the veins can be seen agglutinated masses of corpuscles floating in clear fluid plasma.

Ventricles and auricles filled with pale clot.

Dog VI.—Weight 4½ lbs.

10 a.m., 2nd March 1908.—Received 3 c.c. goat's serum subcutaneously.

3 a.m., 2nd March 1908.—Isotonic point . . . . . 475 salt.

10 a.m., 3rd March 1908.—Seen to be passing hæmoglobinous urine.

Dog killed.

Autopsy—

Spleen enlarged, black in colour, firm consistency, surface tuberculated.

Kidneys dark purple in colour.

Hæmoglobinous urine in bladder.

Hæmoglobinæmia in all situations.

Blood corpuscles from hepatic vein at 37 in ·9 per cent salt—slight hæmolysis.

Blood corpuscles from engorged spleen at 37 in ·9 per cent. salt—marked hæmolysis.

Microscopical appearance—

Spleen. Phagocytosis of unaltered red cells extremely marked.

A count of 500 cells gave:—

Small phagocytes	unaltered red cells	·4 per cent.
	altered red cells	·4 „
Large macrophages containing many altered and many more or less unaltered red cells		·6 „
Polymorphonuclear cells containing red cells	altered	0
	unaltered	·8 per cent.

Of large mononuclear cells and macrophages 7 per cent. contained red blood corpuscles. Many contained masses of hæmoglobinous material formed from fused red cell substance. Many of the cells were so packed as to resemble masses of hæmoglobin.

Liver—

Small phagocytes 5 per cent. of total cells not including liver cells.

Large macrophages 2 per cent. of total cells not including liver cells.

Lungs—

Extensive engulfment of red cells. The small macrophages and molymorphonuclear cells containing red blood corpuscles being especially noticeable. A count of 300 cells gave:—

Small phagocytes containing red cells	·3 per cent.
Large macrophages containing red cells	3·7 „

Polymorphonuclear cells containing red blood corpuscles were also frequent.

This dog showed that the capillary system of the lungs is the site of very considerable engulfment of red cells.

Dog VII.—Weight 4 lbs.

10 a.m., 2nd March 1908.—Received 1 c.c. of goat's serum subcutaneously.

11 a.m., 5th March 1908.—Dog for the last few days has been getting more and more seedy with progressive anæmia. Weakness of hind legs as in canine piroplasmosis.

1-30 a.m., 5th March 1908.—Isotonic point has risen so that considerable hæmolysis takes place in ·83 salt. No hæmoglobinæmia.

2 p.m., 5th March 1908.—Gave 1 grain quinine sulphate with sufficient diluted acid to dissolve it. No hæmoglobinuria resulted up to 8 P.M.

8 a.m., 6th March 1908.—Isotonic point between 1 per cent. and ·71 per cent. Dog not so seedy.

8 a.m., 7th March 1908. – Anæmia less marked. Dog improving in health. There is a trace of hæmolysis in ·91 per cent. salt but marked hæmolysis does not occur till the blood was placed in ·525 salt.

Washed corpuscles in ·9 per cent. salt at 37. No hæmolysis in two hours.

11 a.m., 7th March 1908.—Gave 1½ grains quinine sulphate dissolved in diluted acid. No apparent effect.

8th March 1908.— Isotonic point ·71.

9th March 1908.—Isotonic point ·55 red cells; half are nearly all large corpuscles and definitely polychromatic cells of large size average 7 per field.

14th March 1908.—No trace of hæmolysis in ·5 per cent. salt. Red cells normal in appearance.

In spite of a greatly raised isotonic point a dose of quinine sulphate equivalent to a dose of over 30 grains given to a man had no effect in inducing hæmoglobinuria.

Dog VIII.—Weight 5 lbs.

11 a.m., 5th March 1908.—Received 3 c.c. goat's serum.

4-30 p.m., 5th March 1908.—Isotonic point at ·83 per cent. salt. Hæmosozic value ·876.

8 a.m., 6th March 1908.—Isotonic point at 1 per cent. salt. No tint of hæmolysis in 5 per cent. salt.

5 p.m., 6th March 1908.—Isotonic point at ·91. Dog recovering.

8 a.m., 7th March 1908.—Anæmia still marked but much improved. Dog better. Washed corpuscles in 1 per cent. salt at 37. No hæmolysis in two hours.

4 p.m., 7th March 1908.—Polychromatic cells average about 10 per field. Marked contrast between large type of red cell and small round dark cells.

5 p.m., 7th March 1908.—Autopsy. Spleen dark red, weight 7 grammes. Blood from hepatic vein. No hæmoglobinæmia. No tendency to agglutination.

Hæmoglobinuria not detected. Owing to imperfect arrangements for observing the urine it cannot be affirmed with certainty that it did not occur in the night of 5th March 1908.

Dog IX.—Weight 6¼ lbs.

5th March 1908.—Isotonic point below 45 per cent. salt. Hæmosozic value 1·168 per cent. salt.

8 a.m., 6th March 1908.—Isotonic point ·425 per cent. salt.

11 a.m., 6th March 1908.—Received 6 c.c. goat's serum subcutaneously.

5 a.m., 6th March 1908.—Isotonic point unchanged. Dog shows no symptoms or anæmia.

8 a.m., 7th March 1908.—Isotonic point at ·71 per cent. salt. Washed corpuscles in 1 per cent. salt at 37. No hæmolysis in two hours.

10-30 a.m., 8th March 1908.—Isotonic point at ·83 per cent. salt or even a trace ·91 per cent. salt. Dog quiet. Anæmia marked but not profound. Small round corpuscles from the majority of blood cells. Polychromatic cells average 5 or 6 per field.
Gave 1½ c.c. goat's serum.

1-30 a.m., 8th March 1908.—Pallor of tongue greatly increased, dog profoundly ill. Profound anæmia.

5 a.m., 8th March 1908.—Hæmoglobinuria. Plasma tested showed hæmoglobinæmia. Isotonic point showed marked degree of hæmolysis at ·91 per cent. salt.

6 a.m., 8th March 1908.—Autopsy. Bladder full of hæmoglobinous urine resembling that in Black-water Fever. Spleen ½ gram, dark red colour but not black, firm surface not markedly tubercular.

Liver.—Blood in liver obviously richer in corpuscles being of normal blood consistency whereas peripheral blood was extremely watery. Organ not obviously enlarged or markedly congested. Gall bladder full of bile of he colour of iodine liniment.

Kidneys.—Dark purple cortex. Medulla pale.

Lungs.—Colour a bright brick red, some parts being darker than others.

Heart.—No antemortem clot.

Hæmo-Lymph glands.—None detected in this dog though frequently found in other dogs.

Hæmoglobinæmia.—Tints obtained by adding 20 c. m. of blood to ½ c. c. citrate solution compared. The amount in splenic and peripheral blood approximately the same. The tint in the hepatic and renal veins blood is a little deeper.

Ghosts.

Peripheral blood—

Red cells 1,496,000 per c.m. . . . { large cells 42 per cent. / small cells 58 ,,

Ghosts 110,000 per c.m.

Splenic blood—

Red cells 3,080,000 per c.m. . . . { large cells 15 ,, / small cells 85 ,,

Ghosts 308,000 per c.m.

Hepatic blood—

Red cells 6,164,000 per c.m. . . . { large cells 8 ,, / small cells 92 ,,

Ghosts 1,782,000 per c.m.

Renal blood—

Red cells 6,072,000 per c.m. . . . { large cells 13 ,, / small cells 87 ,,

Ghosts 1,144,000 per c.m.

Blood—

From inf. vena cava and heart clot rapidly.

Clot shrinks quickly, leaving rosy red serum. Diluted in same proportion as citrate test employed above gives about the same tint. The rosy red serum in this case is a true indication of the hæmoglobinæmia present.

Hæmosozic value . . ·834 per cent salt.

Liver blood received into its own volume of 5 per cent. sodium citrate—centrifugalised and films made of the deposit shows up especially well the agglutination and the differences between large new cells, small dark cells, and cell shadows.

The blood corpuscles in the hepatic blood were chiefly aggregated into clumps, the size of the clumps varying from those composed of a dozen cells to large masses of 20 or 30. In the spaces between the clumps were many large somewhat polychromatic cells. The centre of the clumps were mainly composed of small dark cells, and there were frequently seen leucocytes which had formed it would seem a nucleus of agglutination.

Ghosts are seen of all sizes, some being slightly larger than red cells, but they are mainly smaller and often evidently composed of portions of cells only. Polymorphonuclears containing red cells are frequent. Cells are as follows.—

	Per cent.
Polymorphonuclears	60
Small mononuclears	17
Transitional	4
Large mononuclears, many with included red cells . . .	11
Macrophages	

Heart blood.—Shows groups of red cells massed about leucocytes. Between these groups and to a large extent free are large and polychromatic cells. The groups, however, are smaller and less conspicuous than in the hepatic blood. Very few examples of red cell engulfment are met with in heart blood.

Splenic Vein.—Large crowded macrophages very conspicuous. Most of the included cells are evidently the small round type. A large proportion of the total cells are leucocytes or splenic cells.

Spleen.—1,000 cells counted gave the following :—

		Per cent.
Small phagocytes	unaltered cells	·7
	altered cells	*Nil.*
Large macrophages with red cells		·6
Polymorphonuclear leucocytes with included red cells		1·7

Occasionally polymorphonuclear leucocytes contain 2 or even 3 red cells. In thicker portions of films large masses of fused red cells are seen, and many of the macrophages almost consist of this material held together with a minimal amount of protoplasm.

Lung.—Red cells seen mainly of small dark type, large and polychromatic cells not at all conspicuous. Mononuclear cells with green pigment very noticeable.

Bone Marrow.—Little evidence of cell engulfment. A few included cells chiefly in large mononuclear leucocytes seen on searching.

Kidney.—Substance resembling hæmoglobin in various sized globules from minute droplets to bodies half the diameter of a red cell scattered throughout films. They are mainly derived from the ruptured epithelial cells of the kidney tubules many of which are loaded with similar granules.

Dog X.—Weight 4¼ lbs.

7th March 1908.—Isotonic point ·475 per cent. salt.

5 p.m. 13th March 1908.—Received 3 c.c. goat's serum.

10 a.m., 14th March 1908.—At site of injection but little reaction. No noticeable anæmia. Temperature 100. Polychromatic or large cells about 1 per field. Many small dark cells.

Isotonic point ·59 per cent. or possibly ·71 per cent. salt.

15th March 1908.—Isotonic point ·9 per cent. salt. Dog not very ill but there is marked pallor of the tongue and a considerable amount of anæmia.

Temperature 101·8.

1 p.m., 15th March 1908.—Isotonic point ·91 per cent. salt. No hæmoglobinuria.

9 p.m., 15th March 1908.—Isotonic point ·83.

9 p.m., 16th March 1908.—Dog much better. Isotonic point ·83.

9 p.m., 7th March 1908.—Blood received into 3 c.c. 1 per cent. salt to which one drop of goat's serum was added left in incubator 15 minutes; no hæmolysis. It was then centrifugalised and isotonic point of—

Corpuscles—·525 salt = no hæmolysis
·375 „ = marked hæmolysis
} Accurate observation interfered with by agglutination.

Placed in incubator in 1 per cent. salt agglutinated corpuscles do not hæmolyse.

Dog XI.—Weight 3½ lbs.

5 p.m., 13th March 1908.—Received 6 c.c. goat's serum.

10 *a.m., 14th March 1908.*—Swelling at site of injection slight. There is some pallor of tongue and some anæmia. Temperature 101·2.

Isotonic point is ·91. Polychromasia about 5 per field, cells very irregular in shape, small badly displayed though film is a good one. Tendency to form masses of 20 to 50 cells, the majority being the small round variety.

1 p.m., 14th March 1908.—Passed hæmoglobinous urine, Hæmoglobinæmia not observable in $\frac{1}{100}$ dilution, but seen on using larger quantities of blood.

Isotonic point is ·9 per cent. salt, but in place of "h" it shewed only "tr."

4 p.m., 14th March 1908.—Isotonic point is ·91, but shows trace only at both ·91 and ·83. Some more urine passed, but contains less hæmoglobin than last.

Autopsy—

Tissues pale.

Spleen.—10 grams. Blackish colour. Pulp tarry. Surface with tuberculations.

Liver.—Dark in colour. Weight 75 grams.

Contains blood much richer in red cells than that in the organ generally.

Portal and renal veins and inf. vena engorged.

Kidneys dark purple. Medulla pale.

Lungs.—Brick red.

A few hæmolymph glands seen, the largest not more than the size of a lentil.

Bladder contains dark urine not very hæmoglobinous.

Hæmoglobinæmia present in all situations. Most marked in renal and hepatic veins, especially the former; but only a trace visible in 10 dilution.

Hepatic blood—
Red cells 2,904,000 per c.mm. . . Only 2 per cent. large cells.
Ghosts 88,000 per c.mm.

Renal blood—
Red cells 1,822,000 per c.mm. . . Very few large cells.
Ghosts 176,000 per c.mm.

Portal blood—
Red cells 1,000,000 per c.mm. . . Only 2 per cent. large cells.

Femoral blood—
Red cells 836,000 per c.mm.

In 1,000 cells counted ghosts were encountered as follows :—

Portal	21 ghosts.
Hepatic	26 ,,
Splenic	7 ,,
Femoral	10 ,,
Renal from portions of kidney	95 ,,
Pulmonary	15 ,,
Renal from renal vein	70 ,,

(1) Washed blood corpuscles from portal vein + serum of normal dog = no hæm olysis or agglutination.

(2) Washed corpuscles from portal, hepatic, and renal veins and from ventricles left in sugar and salt solution at 37° C., no hæmolysis or agglutination.

(3) Washed portal blood corpuscles + crushed liver substance = marked agglutination, no hæmolysis.

(4) Washed spleen pulp in sugar and salt solution at 37° C. no hæmolysis, doubtful agglutination.

Hæmosozic value is about ·938 per cent. salt.

Centrifugalised renal blood shows tendency to form agglutinated masses with free polychromatic cell.

Centrifugalised portal blood showed a good deal of red cell phagocytosis.

Dog XII.—Weight 4¾ lbs.

5 p.m., 13th March 1908.—Received 3 c.c. goat's serum 10 c.c. normal serum.

10 a.m., 14th March 1908.—Site of injection of 3 c.c. specific serum much swollen.
Site of injection of 10 c.c. normal serum not nearly so markedly swollen.
No noticeable pallor of tongue. Anæmia swollen moderate.
Isotonic point ·59 per cent. salt.
Large and polychromatic cells about 1 per field, many small round cells.

4 p.m., 14th March 1908.—Temperature 100·4. Extremities cold.

9 p.m., 14th March 1908.—Isotonic point ·83 per cent. salt.

1 p.m., 15th March 1908.—Isotonic point ·91 per cent. salt, slight hæmolysis at ·83 per cent. salt.

5 p.m., 15th March 1908.—Received 3 c.c. goat's serum.

16th March 1908.—Blood very anæmic. Dog very seedy. Isotonic point ·91 per cent. salt; more marked hæmolysis at ·83 per cent. salt.

4 p.m., 16th March 1908.—Blood shows marked hæmolysis in ·91 per cent. salt and there is a trace of free hæmoglobin in the serum. Shortly after dog passed hæmoglobinous urine.

Autopsy—

Urine in bladder lighter than that passed shortly before.

Spleen.—11 grams, dark colour, not very tuberculated.

Engorgement of visceral veins, portal, renal, and inf. vena cava very noticeable.

Blood from different sources diluted 5 times with 5 per cent. citrate solution and centrifugalised showed—

Splenic	tint.
Portal	tint.
Hepatic	slightly darker tint.
Renal	marked red tint.

The number of red cells in blood from different sources counted in the Thoma-Zeiss apparatus gave:—

Splenic Vein—	
Large cells	149,600
Small cells	127,600
Ghosts	13,200
Renal Vein—	
Large cells	40,000
Small cells	61,600
Ghosts	127,600
Portal Vein—	
Large cells	528,000
Small cells	13,200
Ghosts	4,400
Hepatic Vein—	
Large cells	448,800
Small cells	136,400
Ghosts	8,800

Difference between large and small cells very striking.

The significance of these experiments will be discussed in the following chapter.

Chapter III.

General Consideration of Blood Destruction in Relation to Hæmoglobinuria.

Levadite (76), studying the result of the injection of specific hæmolytic sera concludes that the principal cause of the anæmia in animals so treated is an intense phagocytosis of the red cells in the spleen. The fact that such an intense phagocytosis takes place will be evident from a study of our experiments upon dogs. It is even more important in our present connection to note that hæmoglobinæmia was not necessarily associated with this form of blood destruction.

As soon, however, as hæmoglobinæmia came about hæmoglobinuria followed. In these experiments then the hæmoglobinuria was no mere measure of the amount of hæmoglobinæmia, but an indication that such a condition had supervened.

This conclusion is supported by our observations upon Black-water Fever, for in this disease hæmoglobin is excreted in the urine even when hæmoglobinæmia is scarcely to be detected by the spectroscope. Hunter (68) also comes to the conclusion that hæmoglobinæmia in the peripheral circulation however small in amount leads to hæmoglobinuria. On these grounds it appears reasonable to conclude that hæmoglobinuria and hæmoglobinæmia are concomitant phenomena, the former necessarily following the appearance of the latter. Against this view there are the observations of Ponfick (69) who found that on injecting laked horses blood into dogs no hæmoglobin was excreted until the amount free in the circulation reached one-sixtieth of the total hæmoglobin of the body. But that these experimental data do not apply in Black-water Fever is certain, since hæmoglobinuria commonly occurs as we have seen with an amount of hæmoglobinæmia much less than would be caused by the presence of one-sixtieth of the total hæmoglobin, and may be present when the amount of hæmoglobin in the plasma is so small as to escape detection.

Again in many conditions hæmoglobinæmia has been described as present without the occurrence of hæmoglobinuria; but these observations seem all to refer not to the recognition of hæmoglobin in solution in the plasma, *i.e.*, true hæmoglobinæmia, but to the presence of hæmoglobin in the serum exuded from the blood after this has clotted.

Such observations as we have previously pointed out are open to grave error. They may relate, indeed, to quite a distinct condition, the recognition of which is in itself of great importance, namely, that, on clotting of the blood, solution of a certain number of red cells has occurred. Murri and Justus allowed blood

obtained by venesection from thirty syphilitic patients to clot at a high and a low temperature. In the former twenty-eight out of the thirty showed a reddish serum, but in all the latter a clear serum exuded. Here then the reddish serum was not an indication of the existence of true hæmoglobinæmia. Other examples are given in the case of dogs 1 and 2 (*vide* experiments).

Until therefore we have observations specifically noting the presence of hæmoglobinæmia in the plasma as against its presence in the serum after clotting we are unable to judge whether in such cases true hæmoglobinæmia unassociated with hæmoglobinuria is present.

Another very important point emphasized by these experiments is that until hæmoglobinuria supervened, the most severe blood destruction was unassociated with the presence of hæmoglobinæmia. Even very large doses of the hæmolytic serum though they produced a correspondingly more rapid and intense anæmia did not on this account give rise to hæmoglobinæmia, and the anæmia seemed due almost entirely to removal from the circulation of damaged corpuscles and their eventual engulfment by macrophages. Since hæmoglobinæmia does not necessarily result from the most intense blood destruction its appearance must depend upon some new factor or super-added condition. This new condition seems to us to be the solution of the red cells in the plasma, in contra-distinction to their retention in the organs, and their ultimate phagocytosis and intracellular digestion. Therefore we have two forms of blood destruction to consider.

(1) The holding up and immediate, or ultimate, phagocytosis of red cells, apparently unassociated with any extra-cellular solution.

This method of red cell destruction we propose to call for convenience of description "ERYTHROCATALYSIS."

(2) That condition, in which the red cells undergo solution in the plasma, and in which results true hæmoglobinæmia followed by hæmoglobinuria.

This condition we propose to call "LYSÆMIA."

The condition met with where true hæmoglobinæmia is not present, but where the blood after clotting shows the presence of hæmoglobin dissolved in the serum may be appropriately named, "EXTRA-VASCULAR LYSÆMIA."

I.—Erythrocatalysis.

The condition seen in the spleen and other organs of animals inoculated with specific hæmolytic serum resembled in a remarkable degree that which we have already described in the spleen and liver of Black-water Fever.

The cells in which ingested red blood corpuscles are seen have the same general characters as those seen in Black-water Fever. They are:

(1) Comparatively small cells containing a single red corpuscle, which is generally either normal in appearance or only slightly altered. These cells possess the same crescentic nuclei and scanty protoplasm, just sufficient to surround the engulfed red cell, already noted in those seen in Black-water Fever.

(2) Large cells evidently of the same type, but containing many more red cells exactly resembling those described in the spleen in Black-water Fever.

Besides being present in the splenic pulp and liver capillaries, cells of this nature were extremely abundant in the blood from the splenic and hepatic veins. They appear to be actively engaged in transporting engulfed red cells. As large numbers are present in the hepatic blood, but are not seen in the peripheral circulation, it seems probable that many of them are stopped in the capillary system of the lungs.

Many cells of this type are found surrounding dense masses of red cell substance. The nucleus is pushed to one side and the protoplasm forms a scarcely perceptible envelope. They more nearly resemble masses of hæmoglobin than cells.

(3) Endothelial plaques which often consist of several imperfectly differentiated cells. They are almost certainly derived from the visceral (splenic and hepatic) endothelium.

Like the last mentioned cells they are common in the splenic and hepatic veins and occur occasionally in the peripheral blood.

They contain *débris* of various kinds, including altered and unaltered red cells, vacuoles and fragmenting masses of nuclear matter or engulfed leucocytes.

(4) *Polymorphonuclear cells.*—These are actively engaged in engulfing red cells and may be seen with one, two or even three included cells.

We have not seen this type of cell in active red cell phagocytosis in Black-water Fever.

The included red cells are often at first sight quite normal in appearance. But a closer inspection shows that many are a trifle paler than normal, while others are smaller and darker. Most of the cells become eventually clear vacuoles, which later become contracted to smaller and smaller dimensions.

But in some case the engulfed red cells takes on a greenish tinge, and gradually becomes converted into a dull greenish mass; this latter change is, however, not very frequently seen.

Engulfment is most active in the spleen, it is somewhat less so in the liver capillaries; and is but little in evidence in the kidneys and bone marrow.

In the lung capillaries it was very conspicuous, and some of them were filled with rows of macrophages and polymorphonuclear cells containing engulfed red cells.

Erythrocatalysis in Black-water Fever is not then very noticeably different from that seen in the experimental conditions we have described.

II.—Lysæmia.

We have previously drawn attention to the fact that once hæmoglobinæmia is established hæmoglobinuria follows. In order to ascertain the site of this form of hæmolysis, observations were undertaken in regard to—

(1) The existence of stromata,

(2) The degree of hæmoglobinæmia,

in different parts of the circulation. A peculiar point at once became evident, namely, that, whilst the peripheral blood was intensely anæmic, that in the liver and kidneys and to a less extent the spleen was much richer in corpuscles. This is very evident from the counts given for dogs (11 and 12).

Though stromata were occasionally seen in the peripheral blood and in the spleen, they were more numerous in the portal and pulmonary veins; they were most numerous of all in the kidney and liver. It will be seen from the experiments given in full at the beginning of this chapter, that the amount of hæmoglobinæmia followed very closely this distribution, being greatest in the blood of the hepatic and renal veins. The accumulation of shadows in the organs may occur, however, without it necessarily following that they have been produced in this situation, and the concentration of the blood in the liver and kidneys may also account for the slightly greater amount of hæmoglobinæmia seen in the blood from this portion of the circulation. The evidence of shadow corpuscles and the amount of hæmoglobinæmia therefore suggests though it does not prove these organs to be mainly concerned; and the fact of greater concentration of the blood in these situations is also significant.

The agglutination of corpuscles so noticeable in the blood from the hepatic vein seems at any rate to show that it was chiefly in passing through this organ that the blood was acted upon by agglutinins which were present in the specific serum employed.

III.—The Effect of the Injection of Hæmolytic Serum and Snake Venom upon the Isotonic Point.

To explain hæmolysis in certain diseases, reduced resistance of the red cells has often been hypothecated; and this reduced resisting power has been very

generally considered as bearing a more or less definite relation to their resistance to laking by salt solutions; or in other words to be associated with alterations in their isotonic point.

Variations in the isotonic point have been described by Vicarelli, Limbeck (80), Castellino, and others, and this point may reach in certain pathological conditions ·66 % salt solution (normal ·46 %, *vide* page 124).

We are not aware that any observer has assigned a definite reason for these changes, so that even apart from any relation the discovery may have to our present studies, it is a matter of considerable importance and interest that in dogs and rabits a raising of the isotonic point to ·7 %, ·8 %, or even ·9 % salt solution is observed as a result of the injection of specific hæmo-toxins.

The following observations will make this apparent :—

Dog 4. 15 c.c. goat's serum injected 10 A.M. 2nd March 1908.

Percentage salt.	1·00	·91	·83	·77	·71	·66	·59	·55	·525	·50	·475	·45	·425	·4	
27th February 1908 . .	...	...	...	...	...	*Nil*	*Nil*	*Nil*	tr	h	inc H	inc H	...	..	.....
1 P.M., 2nd March 1908 .	*Nil*	*Nil*	*Nil*	tr	h	hh	inc H	inc H	inc H	..	...	...	...	.	Dog very ill. Shivering.
2 P.M., 2nd March 1908 . .	*Nil*	*Nil*	tr	h	h	...	...	..	...	...	...	...	...	...	Marked anæmia. No hæmoglobinæmia Dog killed.

Dog 7. 1 c.c. goat's serum injected 10 A.M., 2nd March 1908.

Percentage salt.	1·00	·91	·83	·77	·71	·66	·59	·55	·525	·50	·475	·45	·425	·4	
1-30 P.M., 5th March 1908 .	*Nil*	*Nil*	hh	inc H	inc H	inc	...	...	...	..	..	...	...	..	Dog ill. Marked anæmia. No hæmoglobinuria.
8 A.M., 6th March 1908 . .	*Nil*	*Nil*	hh	inc H	inc H	...	...	...	...	..	...	...	.	...	Ditto.
7th March 1908 . ..	*Nil*	tr	h	hh	hh	hh	hh	hh	inc H	...	.	...	...	..	Dog not so ill.
8th March 1908 . .	*Nil*	*Nil*	h	hh	hh	hh	hh	hh	hh	hh	inc H	inc H	...	...	.....
11 A.M., 9th March 1908 . .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	tr	h	h	hh	inc H	inc H	...	Corpuscles almost all very large or polychromatic.

NOTE.—tr=trace; h=slight hæmolysis; hh=more marked hæmolysis; inc H=incomplete hæmolysis; H=complete hæmolysis.

Dog 8. 3 c.c. goat's serum injected 11 A.M., 5th March 1908.

Percentage salt.	1·00	·91	·87	·71	·66	·59	·55								
4-30 P.M., 5th March 1908 .	*Nil*	*Nil*	tr	hh	inc H	inc H	...	...	...	...	...	...	...	...	.....
8 A.M., 6th March 1908 . .	tr	h	inc H	inc H	...	...	...	...	...	...	...	...	...	...	Hæmoglobinuria during night of 5th March 1908.
5 P.M., 6th March 1908 . .	*Nil*	tr	h	inc H	...	...	...	...	...	...	...	...	...	...	.....
8 A.M., 7th March 1908 . .	*Nil*	tr	h	inc H	inc H	...	...	...	...	...	...	...	...	...	.....
					In 5 per cent. salt, no trace of tint.										

Dog 9. 6 c.c. goat's serum injected 11 A.M., 6th March 1908.
1·5 c.c. „ „ „ 10-30 A.M., 8th March 1908.

Percentage salt.	1·00	·91	·83	·71	·66	·59	·55	·525	·50	·475	·45	·425	·4	·375	
5 P.M., 6th March 1908 . .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	h	inc H	.....
8 A.M., 7th March 1908 . .	*Nil*	*Nil*	*Nil*	h	inc H	inc H	...	...	...	...	...	...	...	...	.....
10-30 A.M., 8th March 1908 .	...	tr	h	hh	inc H	inc H	inc H	...	...	...	...	...	...	...	.....
5 P.M., 8th March 1908 . .	tr	h	h	inc H	inc H	...	...	...	...	...	...	...	...	...	Hæmoglobinuria. Hæmoglobinæmia.

Dog 10. c.c. goat's serum injected 5 P.M., 13th March 1908.

Percentage salt.	1·00	·91	·83	·71	·59	·50	·475	·425							
7th March 1908 . .	*Nil*	*Ni*	*Nil*	*Nil*	*Nil*	*Nil*	h	inc H	...	...	...	...	..	...	.....
10 A.M., 14th March 1908 .	*Nil*	*Nil*	*Nil*	*Nil*	h	hh	..	...	...	...	...	...	...	...	Many spherocytes.
9 P.M., 14th March 1908 . .	*Nil*	tr	h	hh		...	...	...	...	...	...	...	...	...	No hæmoglobinuria.
1 P.M., 15th March 1908 .	*Nil*	tr	tr	hh	in H	...	..	...	...	...	...	...	...	...	Ditto.
9 P.M., 15th March 1908 .	*Nil*	*Ni*		h	...	...	...	...	...	...	...	...	...	...	Ditto.
16th March 1908 . .	*Nil*	*Nil*	tr	h	...	...	...	...	...	...	...	...	...	...	Ditto.

Dog 11. 6 c.c. goat's serum injected 5 P.M., 13th March 1908.

Percentage salt.	1·00	·91	·83	·77	·71										
8 A.M., 14th March 1908 .	*Nil*	h	inc H	inc H	...	...	...	...	...	...	...	...	...	...	.....
1 P.M., 14th March 1908 .	tr	h	h	inc H	...	...	...	...	...	...	...	...	...	...	Hæmoglobinuria.
4 P.M., 14th March 1908 .	tr	h	hh	H	...	...	...	...	...	...	...	...	...	...	Hæmoglobinuria.

Dog 8. 3 c.c. goat's serum injected 11 A.M., 5th March 1908.

Percentage salt.	1·00	·91	·87	·71	·66	·59	·55								
4-30 P.M., 5th March 1908 .	*Nil*	*Nil*	tr	hh	inc H	inc H	...	...	...	...	...	...	...	...	.....
8 A.M., 6th March 1908 . .	tr	h	inc H	inc H	...	...	...	...	...	...	...	...	...	...	Hæmoglobinuria during night of 5th March 1908.
5 P.M., 6th March 1908 . .	*Nil*	tr	h	inc H	...	...	...	...	...	...	...	...	...	...	.....
8 A.M., 7th March 1908 . .	*Nil*	tr	h	inc H	inc H	...	...	...	...	...	...	...	...	...	.....
					In 5 per cent. salt, no trace of tint.										

Dog 9. 6 c.c. goat's serum injected 11 A.M., 6th March 1908.
1·5 c.c. „ „ „ 10-30 A.M., 8th March 1908.

Percentage salt.	1·00	·91	·83	·71	·66	·59	·55	·525	·50	·475	·45	·425	·4	·375	
5 P.M., 6th March 1908 . .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	h	inc H	.....
8 A.M., 7th March 1908 . .	*Nil*	*Nil*	*Nil*	h	inc H	inc H	...	...	...	...	...	...	...	...	.....
10-30 A.M., 8th March 1908 .	...	tr	h	hh	inc H	inc H	inc H	...	...	...	...	...	...	...	.....
5 P.M., 8th March 1908 . .	tr	h	h	inc H	inc H	...	...	...	...	...	...	...	...	...	Hæmoglobinuria. Hæmoglobinæmia.

Dog 12. 3 c. c. goat's serum *plus* 10 c. c. normal goat's serum injected.
3 c. c. goat's serum injected 5 P.M., 15th March 1908.

Percentage salt.	1·00	·91	·83	·77	·71	·66	·59	·55	·50						
8 A.M., 14th March 1908 . .	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	tr	h	hh	...	...	...	...	...	.....
4 P.M., 14th March 1908 . .	*Nil*	*Nil*	tr	tr	h	h	h	...	...	...		...	...	...	No hæmoglobin in urine.
9 P.M., 14th March 1908 . .	*Nil*	*Nil*	tr	...	h	.	...	...	...	...	...	..	...	..	Ditto.
4 P.M., 15th March 1908 . .	...	tr	h	inc H	inc H	...	...	...	...	...	...	...	...	...	Ditto.
5 P.M., 15th March 1908 . .	*Nil*	*Nil*	tr	h	...	...	...	...	...	...	...	...	...	..	Ditto.
8 A.M., 15th March 1908 . .	*Nil*	tr	hh	inc H	inc H	...	...	...	...	...	...	...	...	...	Ditto.
4 P.M., 16th March 1908 . .	tr*	hh	inc H	...	..	..	...	...	...	...	...	...	...	...	Hæmoglobinuria and hæmoglobinæmia.

* Trace also in 5 per cent. salt.

In order to see whether this effect, clearly demonstrated upon dogs, was of general application, we used another species of animal, namely, rabbits. As we had not at hand a specific serum hæmolytic for rabbits blood we utilised (1) daboia venom, (2) cobra venom lecithid. The results of these experiments in regard to the isotonic point were as follows :—

Experiments with daboia venom.

Rabbit I.—Weight 3 lbs.

4 P.M. Received 50 m. grms. dried daboia venom in solution.
6 P.M. Animal breathing rapidly. Cyanosis of ears.
9 P.M. Animal died.

ISOTONIC POINT.

	·91 %	·83 %	·17 %	·62 %	·52 %	·45 %	+ %
4 P.M. .	...	...	o	o	trace	hh	complete
6 P.M. .	...	...	o	o	marked	nearly complete.	complete
9 P.M. .	o	o	nearly complete.	nearly complete.	...	...	...

No hæmoglobinuria.

Rabbit II.—Weight 2½ lbs.

8-30 A.M. Received 25 m. grms. dried daboia venom in solution.
11 A.M. Died.

ISOTONIC POINT.

	62 %	·32 %	·45 %	·425 %	·4 %
8 A.M.	...	...	o	h	nearly complete.
11 A.M. (just before death) . .	o	marked	nearly complete.	nearly complete.	nearly complete.

No hæmoglobinuria.

Rabbit III.—Weight 2½ lbs.

8-30 A.M. Received 10 m. grms. dried daboia venom in solution.
11 P.M. Died.

ISOTONIC POINT.

	·82%	·71 %	·62 %	·52 %	·45 %	·425 %	·4 %
8 A.M. .	0	0	0	0	0	tr	nearly complete.
4 P.M. .	0	tr	nearly complete.	nearly complete.	nearly complete.	...	...

No hæmoglobinuria.

Experiments with cobra venom lecithid.

Rabbit No. 1.—Weight 3 lbs. At 8-30 A.M. ·01 grm. cobra.
At 4 P.M. ·15 grms. lecithid.

No noticeable effect produced upon the animal.

ISOTONIC POINT.

	·71 %	·62 %	·52 %	·45 %	·4 %
12 A.M.	0	0	tr	h	complete.
4 P.M.	0	0	0	tr	complete.
9 P.M.	0	0	0	0	nearly complete.

Rabbit No. 2.—Weight 2¼ lbs. At 8-30 A.M. ·03 grm. cobra lecithid.
At 4 P.M. ·3 grm.

Animal showed no ill effects.

ISOTONIC POINT.

	·71 %	·62 %	·52 %	·45 %	·4 %
12 A.M.	...	0	0	tr	complete.
4 P.M.	.	0	h	hh	complete.
9 P.M.	...	0	0	0	nearly complete.

The raising of the isotonic point seems therefore to be a frequent concomitant or result of specific hæmotoxic action on the red cells. The absence of any change in the case of the non-toxic cobra venom lecithid we shall refer to in Part IV.

An important deduction to be made from these results is that, if a raised isotonic point be shown in Black-water Fever, it would not necessarily show lysæmia to be the result of changes in the osmotic relations of the plasma and corpuscles, but it would be presumptive evidence of the presence of a hæmotoxin.

IV.—Relation of Isotonic Point to Lysæmia.

It has been noticed that by the injection of specific serum or snake venom, the isotonic point of the red corpuscles is raised in varying degree. It must also be remarked that when this raising of the isotonic point had reached a certain value, *i.e.*, about ·9 per cent. salt solution, hæmoglobinuria resulted; so long however as the isotonic point remained below this, animals exhibited neither hæmoglobinæmia nor hæmoglobinuria.

Several estimations gave the hæmosozic value in dogs at about equal to ·9 per cent. to 1 per cent. salt solution. It seemed therefore possible that, in these experiments, lysæmia was the result of the solution of red cells whose power of resistance to hypotonic salt solution was *nil*, and occurred at the time when the isotonic point had reached the hæmosozic value of the plasma.

Another interesting point in regard to the isotonic changes in experimental hæmoglobinuria is seen in animals that have recovered from the effects of hæmolytic serum. In such cases the actual isotonic point, *i.e.*, the salt solution, just insufficiently weak to cause any hæmolysis, remains high for two or three days, whilst the amount of hæmolysis in the upper tubes of the series becomes less and less, so that before any considerable laking takes place one has to descend nearly to the normal salt value. Finally quite suddenly one day the slight hæmolysis seen in the upper tubes disappears. In such cases it is easy to see that the phenomenon is due to the existence of two types of red cells, a diminishing number of dark small corpuscles and an increasing number of large and more or less polychromatic cells. With the disappearance of the former the isotonic point of the blood returns to normal or nearly so (*vide* Dog VII).

These experiments show that the large polychromatic cells are not degenerated corpuscles, but the newly formed ones.

Their appearance seems therefore a phenomenon exactly similar to the output of polychromatic and large cells after an attack of Black-water Fever.

It is not difficult to understand that the slight decrease noted by us as sometimes seen in the isotonic point of Black-water Fever blood may be due to this outpouring of new corpuscles into the circulation, rather than to the more resistant cells having been left untouched by the blood-destroying process, the explanation usually offered.

Chapter IV.

The Lysæmia of Black-water Fever.

The facts we have brought forward throw a good deal of light upon the nature of the Black-water Fever process. It becomes evident that it is not the mere amount of blood destruction but the nature of the process that is the fundamental factor in the causation of the hæmoglobinuria of Black-water Fever. Viewed in this light it is no longer difficult to see why in the severest attacks of malaria, although blood destruction may be very great, hæmoglobinuria does not result. The loss of red cells in an acute initial attack of malaria may amount according to Kelsch (73) to 1,000,000 per c.m. in one day, and Dionisi (73) has observed a fall of 500,000 per c.m. in twelve hours; yet these cases showed no hæmoglobinuria. On the other hand cases of hæmoglobinuria have been observed in which the maximum loss of red cells amounted only to 129,000 per c.m. (Bristowe and Copeman). In malaria we have an example of what we have termed "erythrocatalysis"; in Black-water Fever the process is essentially a lysæmia.

In malignant tertian malaria some of the most noticeable features of the condition are—(*a*) the tendency to accumulation of infected corpuscles in the visceral capillaries, (*b*) the excessive rarity of sporulating bodies in the peripheral circulation, and (*c*) the frequently recorded marked alteration in he infected red cells and active engulfment of these by phagocytes. All these peculiarities are evidently due to the specially pronounced action of the malignant tertian parasite in bringing about erythrocatalysis.

In quartan and benign tertian malaria, on the other hand, there is not the same tendency for infected red cells to be held up in the visceral capillaries; segmenting parasites, especially in the case of quartan infection, appear very frequently in the peripheral circulation, and phagocytosis of infected red cells is so rare that Thayer states that he has never observed it. From such considerations we should judge that the quartan parasite had an effect even feebler than that of the benign tertian parasite in bringing the infected corpuscle to the state in which it becomes subject to erythrocatalysis.

In pernicious anæmia according to Hunter there is solution of red cells in the portal circulation, due to the action of hæmolytic substances absorbed from the gastro-intestinal tract; in other words, a portal lysæmia takes place. Whether in Black-water Fever the lysæmia occurs in the portal vein, but in such large amount as to produce general hæmoglobinæmia; or whether the hepatic or peripheral circulation forms the site of the process we do not know.

It still remains for us to ascertain, if possible, the actual nature of the lysæmia of Black-water Fever, whether it is due to change in the osmotic relations, chemico-toxic bodies, or to the production within the body of some specific hæmolysin.

Black-water Fever not due to changes in osmotic relations of the red cells and plasma.

Hamburger (73), Von Limbeck (80), and others have studied the behaviour of red blood corpuscles in salt solutions of different strengths. If blood corpuscles be placed in salt solutions weaker than the plasma in which they have been bathed they do not necessarily become destroyed; only when the salt solution is considerably weaker than the plasma does this happen. Human serum, which for all practical purposes in this connection, may be considered as approximately representing the condition of the plasma, is estimated to have an osmotic tension equal to ·9 per cent. sodium chloride solution (Hamburger). But human red cells become hæmolysed only when a salt solution below about ·46 per cent. salt solution is so used. Such a percentage which is just above the strength of solution necessary to cause laking has been called the "Isotonic point."

It will be obvious that the isotonic point in itself represents no real quality but measures only one kind of resistance of the red cell, the limit of its power to resist want of osmotic relation between its own substance and the plasma.

This resistance is we have just seen so great that for hæmolysis to take place in the body by a reduction in the osmotic tension of the plasma would require, provided the isotonic point remained the same, a reduction of the salt content of the plasma to one-half or more of the normal. This fact explains why under normal circumstances an equal volume of distilled water added to one of blood does not result in marked laking, and also makes it evident that enormous reductions in the salt content of the plasma may take place without any danger of hæmolysis.

But if the power of the red cells to resist laking vary, as indicated by the isotonic point, so that they can stand less osmotic strain, then so much the more is reduction in the salt content of the plasma likely to lead to hæmolysis In investigating the condition of the blood in Black-water Fever it is necessary therefore to consider both corpuscles and plasma, and to estimate both the isotonic point on the one hand, and the hæmosozic value (osmotic tension of plasma) on the other.

Isotonic point. -- In our observations we have estimated the isotonic point by adding measured quantities of blood (10 to 20 c.m.) to known quantities of salt solutions (·5 or 1 c.c.) of various strengths, shaking, allowing to stand for a few

moments, and centrifuging. It is well known that all the blood cells are not of equal isotonicity so that a considerably weaker salt solution is required to produce complete or nearly complete hæmolysis than to show a tint due to solution of the few less resistant corpuscles. In our experiments using fused sodium chloride the first trace of laking in normal blood was generally distinctly visible in a $\frac{N}{13}$ salt solution equal to ·449 per cent. A quite distinct colour was given at $\frac{N}{14}$ or ·417 per cent., and as a rule marked but still incomplete hæmolysis at $\frac{N}{15}$ or $\frac{N}{16}$. Estimates of the percentage of hæmoglobin set free at each dilution of the series were made by comparison with standards, prepared with known percentages of the blood in distilled water.

With slight variations they usually showed as follows :—

$\frac{N}{12}$	$\frac{N}{13}$	$\frac{N}{14}$	$\frac{N}{15}$	$\frac{N}{16}$
Nil.	*Nil.*	5 to 10 per cent.	15 to 30 per cent.	40 to 50 per cent.

In later observations we used a regular series of percentage solutions varying by ·025 per cent. These allowed greater accuracy than normal solutions in which the intervals towards the lower end of the scale are far too wide. Subsequently we adopted for convenience in preparation a series of dilutions prepared from a 10 per cent. solution of sodium chloride.

Every case upon which we have based conclusions has been contrasted with one or more control bloods, and we have also frequently repeated our observations in regard to the more critical points. In Black-water Fever a difficulty is introduced by the intense yellow colour of the serum, and the presence of hæmoglobinæmia giving a tint in all tubes independently of any laking by the salt solution. The anæmia too renders the tints obtained not comparable with those from normal blood, but this difficulty refers only to the amount of hæmolysis in the different tubes; in ascertaining the limit of hæmolysis (isotonic point) and the main facts regarding the behaviour of the blood to salt solutions it does not interfere. A rough allowance for the anæmia was made by comparing the patient's blood with normal in given volumes of distilled water.

As regards the tint due to the colour of the plasma in Black-water Fever, this was allowed for by considering the first increase of tint observed in the series of tubes as equivalent to that produced by $\frac{N}{14}$ salt solution on normal blood.

The isotonic point in Black-water Fever.—Marked changes in the isotonic point have never been found by us in Black-water Fever. By marked changes we refer to such changes as have been given in our animal experiments where

from about ·5 per cent. the isotonic point has been raised to ·7 per cent., ·8 per cent. and even ·9 per cent., when its determination becomes obscured by the existence of hæmoglobinæmia. In Black-water Fever then our researches have been confined to the recording of comparatively minute variations which it must be confessed cannot be considered as affording any appreciable support to the idea that the hæmolysis in this disease has any relation to osmotic variations in the plasma, though they may indicate, as we have seen, the action of hæmotoxins.

Our observations in this connection on Black-water Fever cases are as follows* :—

ISOTONIC POINT OF BLACK-WATER FEVER.

	Strength of salt solution.	N/13	N/13	N/14	N/15	N/16	N/17
Case XVII . .	Four days after onset .	...	...	...	tr	hh	inc H
„ XVIII .	Five days after onset .	...	*Nil*	tr	tr	h	hh
„ XIX . .	Day after onset. Mild case. Hæmoglobinuria over.	*Nil*	*Nil*	h	inc H	...	...
„ XX . .	Day of onset. Hburia .	tint	tint	incr-tint	inc H	...	...
„ „ . .	Day after onset. Hburia .	...	incr-tint	incr-tint	inc H	...	..
„ „ . .	2nd day. Hburia .	tint	tint	h	hh	inc H	...
„ „ . .	3rd day. 6 hours after cessation. Hburia.	*Nil*	*Nil*	h	15% hh	40% inc H	...
	Percentage of salt .	·525	·5	·475	·45	·425	·4
Case XXI . .	6 hours after onset. Severe case.	tint	tint	tint	tint	incr-tint	inc H
„ XXVI .	Day after onset. Hburia .	tint	tint	tint	tint	tint	incr-tint
Control . . .	.....	*Nil*	*Nil*	*Nil*	*Nil*	tr	h
	Percentage of salt . .	·476	·454	·434	·416	·4	·384
Case XXVII .	Day after onset . .	tint	tint	incr-tint	incr-tint	inc H	...
„ „ .	2nd day. Hburia . .	tint	tint	tint	incr-tint	inc H	...
Control . .	.....	*Nil*	*Nil*	*Nil*	h	hh	inc H

*NOTE.—*Vide* footnote on page 114.

Again in cases of malaria we have sometimes seen small variations which may, or may not, have significance in so far as they demonstrate the action of this disease on the blood. Of large variations at all likely to lead to hæmolysis in a condition of even markedly lowered strength of the plasma we have no evidence. In such persons whom we judge to be possible subjects of Black-water Fever in the near future, again we find only changes of extremely small degree. These facts will be clear from the following list of observations :—

ISOTONIC POINT OF EUROPEANS RESIDENT IN THE DUARS.

	Strength of salt solution.	N/12	N/13	N/14	N/15	N/16	N/17
Series 1.							
New-comer . .	Has had no malaria for many years. Control 1 .	*Nil*	tr	h	inc H	inc H	...
New-comer . .	Has had no malaria for a long period. Taking quinine 5 grs. daily. Control 2.	*Nil*	tr	h	inc H	inc H	...
Old resident .	20 years in the Duars .	*Nil*	*Nil*	tr	hh	inc H	...
Resident . .	5 years in the Duars .	*Nil*	tr	h	inc H	...	...
,, . .	,, ,, ,, .	*Nil*	*Nil*	h	inc H	...	...
,, . .	,, ,, ,, .	*Nil*	tr	h	inc H	...	...
,, . .	2 years in the Duars suffering much from fever. Anæmic. Heterogeneity of corpuscles marked.	*Nil*	tr	h	inc H	...	...
,, . .	1 year. Much fever .	*Nil*	*Nil*	h	h	...	...
Series 2.							
Old resident .	After fatiguing journey .	tr	h	hh	inc H	...	...
,, ,, . .	Next morning 10 grs. quinine the previous night.	*Nil*	*Nil*	tr	h	hh	...
,, ,, . .	Jalpaiguri. Healthy conditions.	*Nil*	*Nil*	*Nil*	tr	h	...
Control 2 . .	.....	*Nil*	*Nil*	*Nil*	*Nil*	tr	...

ISOTONIC POINT OF EUROPEANS RESIDENT IN THE DUARS—*contd.*

	Percentage of salt.	·476	·454	·434	·416	·4	·384
Series 3.							
Resident . .	2 years in the Duars .	*Nil*	*Nil*	tr	h	hh	...
,, . .	6 months. Much fever .	*Nil*	*Nil*	*Nil*	tr	h	...
,, . .	6 years	*Nil*	*Nil*	tr	hh	inc H	...
Old resident .	Had Black-water Fever one year ago.	*Nil*	*Nil*	tr	h	inc H	..
Control 1 . .	Some hours before onset of malaria.	*Nil*	tr	h	hh	inc H	...
Resident . .	6 months; taking quinine 5 grs. daily.	*Nil*	*Nil*	*Nil*	tr	...	...
Old resident .	Many years in the Duars .	*Nil*	tr	h	hh	...	...

ISOTONIC POINT OF BENGALI BABUS RESIDENT IN THE DUARS.

	Percentage of salt.	·475	·45	·425	·4	·375
Series 1.						
Bengali Babu .	6 months' resident. Enlarged spleen. 70 per cent. Hb.	*Nil*	tr	h	inc H	...
,, ,, .	6 months' resident 95 per cent. Hb.	*Nil*	tr	h	hh	...
,, ,, .	4 years' resident. 75 per cent. Hb.	tr	h	hh	inc H	...
,, ,, .	Numerous M. T. rings, crescents. Enlarged spleen. 80 per cent. Hb.	*Nil*	*Nil*	tr	h	...
,, ,, .	Many years resident . . .	*Nil*	*Nil*	tr	h	...
,, ,, .	,, ,, ,, . .	*Nil*	*Nil*	h	hh	...
,, ,, .	,, ,, ,, . .	*Nil*	*Nil*	*Nil*	tr	...

ISOTONIC POINT OF SERVANT CLASS.

	Strength of salt solution.	N/14	N/13	N/14	N/15	N/16
Madrasi . .	First attack of fever . . .	tr	tr	h	inc H	
,, . .	Next morning. Fever till 5 A.M. Benign tertian parasites. Had 10 grs. quinine previous night.	*Nil*	tr	h	inc H	...
,, . .	5 days later	*Nil*	*Nil*	tr	hh	...
Control . .	.....	*Nil*	tr	h	inc H	...
	Percentage of salt . . .	·525	·5	·475	·45	·425
Marwari . .	5 years in the Duars. Anæmic. Spleen three inches below costal margin.	*Nil*	tr	tr	hh	inc H

ISOTONIC POINT OF COOLIE CLASS.

	Strength of salt solution.	N/12	N/13	N/14	N/15	N/16
Series 1.						
Coolie . .	Fever. Benign tertian parasites .	*Nil*	*Nil*	tr	h	..
,, . .	Fever. M. T. infection . .	*Nil*	*Nil*	tr	h	...
Control I . .	.....	*Nil*	tr	h	inc H	...
Coolie . .	Anæmia	*Nil*	*Nil*	tr	...	...
,, . .	.....	*Nil*	*Nil*	tr,	...	...
Native child .	8 years old. Anæmia. Fever .	*Nil*	*Nil*	tr	...	...
	Percentage of salt . . .	·475	·45	·425	·4	·375
Series 2.	Jaundice. Serum bright yellow. Bile in urine. A few M. T. rings.	*Nil*	*Nil*	*Nil*	*Nil*	tr
Coolie . .	Anæmia. Enlarged spleen. Many nucleated red cells.	*Nil*	*Nil*	*Nil*	tr	...

ISOTONIC POINT OF COOLIE CLASS.

	Strength of salt solution.	N/12	N/13	N/14	N/15	N/16
Coolie . .	1 year resident. Fever. Enlarged spleen. Numerous S. T. rings.	*Nil*	*Nil*	*Nil*	*Nil*	tr
„ . .	1 year resident. Quartan parasites	*Nil*	tr	h	hh	...
„ . .	Anæmia. Numerous S. T. parasites.	*Nil*	*Nil*	*Nil*	tr	...
Bhutia . .	.....	*Nil*	*Nil*	tr	...	...
Control 2 . .	1 month resident. S. T. parasites	*Nil*	*Nil*	tr	hh	...
Coolie .	Next day after 15 grs. quinine .	*Nil*	*Nil*	tr	hh	...
„ . . .	.....	*Nil*	tr	h	hh	...

ISOTONIC POINT OF AFTER ADMINISTRATION OF QUININE.

The following are observations upon the effect of the administration of quinine sulphate upon the isotonic point :—

Normal person.

Strength of salt solution.	N/12	N/13	N/14	N/15	N/16
Immediately after taking quinine . . .	*Nil*	tr	h	inc H	...
3 hours after administration of quinine sulph. 15 grs. and dil. sulph. acid m. 15.	*Nil*	*Nil*	h	inc H	...
4 hours ditto . . .	*Nil*	tr	h	inc H	...

Resident 5 years. Given 15 grs. quinine sulph. and m. 15 dil. acid sulph.

Strength of salt solution.	N/12	N/13	N/14	N/15
Immediately before taking quinine . . .	*Nil*	tr 6%	h 25%	inc H 40%
5 hours after administration	*Nil*	tr	h	inc H
Next day	...	tr 12%	h 17%	inc H 50%

The action of quinine on the isotonic point, if it exists, is a small one that can have no effect in regard to hæmolysis by osmotic variations.

Hæmosozic value.—Hamburgher estimated the osmotic tension of the plasma by adding small quantities of normal blood corpuscles to various dilutions of the serum to be tested. Later Ruffer (82), Wright and Kelner (83), Wright and Ross (84), McCay (48) and others have studied in greater detail this value which Ruffer has named the "Hæmosozic value" of the serum.

Wright and Kelner's method of estimating the hæmosozic value, which is also used by McCay, is briefly as follows:—

In a number of capillary tubes a series is made by mixing 1 vol. of blood with 2 vols. of different salt solutions of greater and greater dilutions and noting at which point hæmolysis takes place. The serum to be tested is then diluted until 1 vol. blood *plus* 2 vols. of the diluted serum gives hæmolysis.

It is obvious that the serum diluted to the extent noted is supposed to act in the same manner as the particular strength of salt solution determined in the first estimation.

If for example:—

1 vol. blood + 2 vol. $\frac{N}{30}$ salt just gives hæmolysis and 1 vol. blood + 2 vols. $\frac{\text{serum}}{\text{diluted 5 times}}$ just gives hæmolysis,

then $\frac{\text{Serum}}{4}$ = same strength as $\frac{N}{30}$ salt solution;

or $\frac{\text{Serum}}{4}$ = ·194,

i.e., the serum = 4 (·194) or ·776 per cent. salt.

In practice there are a number of difficulties in getting accurate results.

1. Want of fixed point as index.—We have already noted that there s no fixed value in regard to the point at which all red cells are hæmolysed.

Thus in $\frac{N}{14}$ or ·417 per cent. the least resistant cells are already hæmolysed.

In $\frac{N}{18}$ or ·324 per cent. there are still many cells undissolved, so that in ascertaining the salt solution in which hæmolysis takes place no fixed point can be determined.

Wright makes use of a change in the colour of the blood as an indication, but this represents no fixed point, because it can be shown by centrifuging that hæmolysis has already commenced before any definite change in the tint of the blood can be observed. McCay uses the point at which complete solution of all cells has supposedly taken place. But this is unsatisfactory owing to the extraordinary persistence of the most resistant cells as shown by the following series of observations:—

1	1 vol. blood	2 vols.	distilled	water:	Hæmolysis	incomplete.
2	"	3 "	"	"	"	"
3	"	4 "	"	"	"	"
4	"	5 "	"	"	"	"
5	"	10 "	"	"	"	complete.

In each case although there was an increasing amount of hæmolysis observable this was incomplete until ten volumes of distilled water had been used.

11.—Wide limit of error.—Since the question of a fall in value of 1 per cent. salt in the osmotic tension of the plasma may be an important point, as suggested by McCay's observations, it appeared highly necessary that we should be able to measure the hæmosozic value within known limits with accuracy, otherwise the uncertainty attaching to our observations would prevent conclusions being based upon them. This has been extremely difficult even in the case of variations as great as ·2 per cent. salt, by the use of either McCay's or Wright's methods. An example of the wide limit allowed by Wright's method will be apparent from the following instance:—

Suppose $\frac{\text{serum } (a)}{6}$ behaves as would an $\frac{N}{28}$ salt solution

and that $\frac{\text{serum } (b)}{5}$ " " " " " "

Then the estimation will give serum (*a*) . . 1·248 per cent.

serum (*b*) . . . 1·040 " "

Difference due to one dilution extra . . . . ·208 per cent.

It is of course possible to make intermediate dilutions; but, taken together with want of fixidity of the index, it is clear that there is much to be desired in regard to delicacy and accuracy. By using a volume of blood with 2 volumes of the different salts, centrifuging and taking the first distinct tint a greater power of detecting differences in the same blood is given, but the most satisfactory and simplest of all methods tried has been a modification of the original method of Hamburgher.

In the isotonic point of one's own blood we possess an index capable of being accurately determined at any moment. Whether one takes the first trace of liberated hæmoglobin or a more marked hæmolysis is immaterial so long as measured quantities of blood and solution are used and the tints compared.

By the use of a sharp spear-pointed lancet or other suitable pricker it is easy to obtain one, two, or even more cubic centimeters of the blood to be tested. This blood is allowed to clot in a suitable tube under conditions which preclude any drying action and the serum is used in preparing a series of dilutions. Of these dilutions $\frac{1}{2}$ c. c. are arranged in small tubes and 10 c. m. of one's own blood added. After centrifugalisation one has a series comparable with another series of one's own blood put up in the ordinary way for the estimation of the isotonic point, thus:—

$\frac{1}{2}$ c.c. of	$\frac{N}{12}$	$\frac{N}{13}$	$\frac{N}{14}$	$\frac{N}{15}$	isotonic series.
$\frac{1}{2}$ c.c. of	$\frac{S}{2}$	$\frac{S}{24}$	$\frac{S}{21}$	$\frac{S}{24}$	serum series.

The index in this case is absolutely fixed; and as one is using a considerable quantity of serum, dilutions of any degree can be made.

Hæmosozic value in Black-water Fever.—The following are estimations of the hæmosozic value in several Black-water Fever cases.

The exact relation to the period of onset, etc., will best be seen by reference to the cases given in the Appendix.

	Per cent.
Case XIX—End of mild attack. Wright's method . .	1·06
Case XX—Onset of severe attack	1·12
Case XX—Next day	1·258
Case XX—Day following	1·25
Case XXI—After onset of severe attack . . .	1·258
Case XXV—Middle of attack	1·14
Case XXVIII—Middle of severe attack. Serum rosy red .	1·05

In Black-water Fever even quite early in the attack notably in Case XX there is no marked if any lowering of the hæmosozic value of the serum below the normal, and certainly no such lowering as could conceivably result in hæmolysis by osmotic tension. That there is not some raising of the hæmosozic value during the attack is less certain.

Neither in the isotonic point nor in the hæmosozic value do we find variations such as might suggest that hæmolysis was due to alterations in the osmotic relation of the corpuscles and the plasma.

Hæmosozic value after quinine administration.—In our earlier estimates we used Wright's method. In many cases no change was obtained, in others there appeared to be some reductionin the value after quinine sulphate administration. Since we dare base no conclusions on these earlier data it is unnecessary to give them.

The following estimations have been made with an unlimited supply of serum, and the results rendered absolutely certain by the greatest care in dilutions and the repetition of the observations.

Estimation by Wright's method modified by blowing out tubes into hypertonic citrate solution centrifuging and comparing tints of the two series.

(1) European. Previously no quinine.

Hæmosozic value before taking quinine 1·168 per cent. salt.

Hæmosozic value 5 hours after administration of—

15 grs. quinine sulph.

15 m. acid sulph. dil. 1·168 per cent. salt.

(2) European. Has not taken quinine for 15 days.

Hæmosozic value before taking quinine 1·4 per cent.

Hæmosozic value 8 hours after administration of—

15 grs. quinine sulph.

15 m. acid sulph. dil. 1·35 per cent.

(3) European. Has not taken quinine for some time.
Hæmosozic value before taking quinine 1·28 per cent.
Hæmosozic value next morning after taking night previous—
10 grs quinine sulph.
10 m. acid sulph. dil. 1·28 per cent.

ESTIMATION BY A MODIFICATION OF HAMBURGER'S METHOD.

Native. Previously no quinine. Blood drawn at 9 A.M. Shortly after 9 A.M. quinine sulphate grains 10 administered. Blood again taken at 5 P.M.

	·5 c.c. dilutions of serum.			·5 c.c. salt solutions.			
	$\frac{S}{2}$	$\frac{S}{2{\cdot}12}$	$\frac{S}{2{\cdot}25}$	·475 per cent.	·45 per cent.	·425 per cent.	·4 per cent.
9 A.M. .	0	trace	distinct	0	0	trace	distinct
5 P.M. .	0	trace	distinct	0	0	trace	distinct

Next morning observations repeated with different blood as an indicator gave exactly the same results with several trials.

Therefore the hæmosozic value equals 2 (·45 per cent.) or ·9 per cent. salt.

European. No quinine for several months previously. Blood taken at 10 A.M. Shortly after, quinine sulphate grains 15 with minims 15 dilute sulphuric acid was administered. Blood taken at 12 A.M., 5 P.M., and next morning.

	·5 c. c. serum dilutions.			·5 c. c. salt solutions			
	$\frac{S}{2{\cdot}2}$	$\frac{S}{2{\cdot}3}$	$\frac{S}{2{\cdot}5}$	·475	·45	·425	·4
10 A.M. .	*Nil*	trace	distinct	*Nil*	*Nil*	trace	distinct

Hæmosozic value equals 2·2 (·45 per cent.) or ·99 per cent. salt.

At	12 A.M.	5 P.M.	Next morning serum gave	
	$\frac{S}{2{\cdot}2}$	$\frac{S}{2{\cdot}2}$	$\frac{S}{2{\cdot}2}$	$\frac{S}{2{\cdot}3}$
	Nil	*Nil*	*Nil*	trace

Hæmosozic value therefore equals 2·2 (·45 per cent.) or ·99 per cent. salt.

We have not therefore been able to find the reduction in the hæmosozic values noted by McCay after the administration of quinine sulphate. These observations are, however, at present few in number, and further work will be required before it can be decided with certainty whether some reduction does not take place. In the meantime there are strong reasons for disbelieving that this supposed action of quinine sulphate has the slightest influence in the causation of Black-water Fever.

That an attack may follow upon administration of the hydrochloride as well as of the sulphate of quinine is shown by reference to the literature. Vedy (96) gives two cases where Black-water Fever occurred after administration of the hydrochloride and three out of the six cases investigated by Werner (58) were after 1 gramme, ·4 gramme and ·6 gramme respectively of quinine hydrochloride.

Again if slight alterations in the salt contents of the plasma are shown sometimes to take place as a result of the ingestion of sulphates, we have no reason to believe that the alteration can extend to such a range of osmotic tension as separates the isotonic point and hæmosozic value in Black-water Fever ; nor are we justified in concluding that such a reduction bears any causal relationship to an attack.

It is of interest to note in this connection that when in one of the experiments on dogs the isotonic point as a result of the injection of specific serum had been raised nearly to the hæmosozic value (*i.e.*, an isotonic point of ·83 per cent. salt solution and a hæmosozic value of about ·9 per cent. salt) a dose of quinine sulphate equivalent in amount to a 30 grain dose in man dissolved in dilute sulphuric acid did not precipitate the onset of hæmoglobinuria (*vide* Dog 7).

Black-water Fever not due to Chemico-toxic action.

The great majority of the chemical hæmolytic bodies give rise to met-hæmoglobinuria. A case of met-hæmoglobinuria in a human subject has been described by Hayem (85).

In this instance the substance giving rise to the condition was unknown, but it was some poison exerting a profound effect upon the red cells, causing met-hæmoglobinæmia and the appearance of this substance together with oxy-hæmoglobin in the urine.

To microscopical examination the red cells appeared small, deformed and brownish in colour and were collected into groups. Decolourised elements were also to be observed, and leucocytes were very abundant, many of them being of great size. In dried films the alterations in the red cells appeared even more marked. They showed fragmentation and abnormal colouration. Polymorphonuclear cells were relatively diminished, but absolutely increased in number. The

proportion of mononuclears was notably increased, this increase being principally due to elements of large size with clear protoplasm. At the same time the blood as a whole was greatly diminished in spite of an absence of excretion. This reduction was also associated with anæmia, the red cells being a little over 1,000,000 per c. m.

The serum showed the spectrum of met-hæmoglobin together with that of oxy-hæmoglobin.

It is evident that though in Black-water Fever a certain amount of met-hæmoglobin may be present in the urine, the process is one of a liberation of oxy-hæmoglobin. Not only is the serum tinted with oxy-hæmoglobin, as shown by the spectroscope, but, if the urine be examined shortly after it is passed, it is quite common to find only the merest trace of met-hæmoglobin ; athough oxy-hæmoglobin is present in abundance.

We can therefore eliminate from among the possible causes of Black-water Fever most chemico-toxic substances; even did the clinical evidence not enable us to do so.

The only substance that it is really necessary to consider in this relation is quinine, which has often been credited as the cause of Black-water Fever.

The hæmolytic effect of quinine hydrochloride has been shown by Stephens and Christophers, who found that it extended to four or five dilutions of a saturated aqueous solution. We have repeated and confirmed this observation, and have made other experiments both with the hydrochloride of quinine and the pure alkaloid.

The latter is extremely insoluble but suspensions in ·9 per cent. salt solution appear to exert no hæmolytic effect upon canine or human red cells.

With regard to the action of saturated solution of quinine hydrochloride the following procedure was adopted. A saturated solution was first prepared containing approximately 3 per cent. (Attfield (86) states that this salt is soluble in 34 parts of water.) This saturated solution was then made up by the addition of sodium chloride until it contained ·9 per cent. of the latter salt.

On the addition of 200 c.ms of this solution to suspensions of 10 c.ms. of human blood in $\frac{1}{2}$ c.c. ·9 per cent. salt solution nearly complete hæmolysis took place, both in the case of a normal and a Black-water blood.

But when in the case of both normal and Black-water blood suspensions, the addition of 150 c.ms. of (*a*) normal and (*b*) Black-water serum was made prior to that of the quinine solution, a reaction occurred accompanied with the precipitation of the quinine as a fine white powder. Prolonged centrifuging failed to entirely clear the supernatant fluid which remained slightly clouded but showed no trace of hæmolysis. In what form quinine may exist in the circulating blood we do not know ; but Stephens and Christophers found that cinchonised serum

taken from a healthy subject shortly after the administration of 2 grms. of quinine when subjective symptoms were present, exerted no hæmolytic action upon the blood of a Black-water Fever case. A consideration of these facts, together with those already referred to in Part I, seems to exclude the action of quinine in itself as being the cause of the lysæmia of Black-water Fever.

The indirect action exerted by this substance in the production of this condition must be left for future consideration.

Black-water Fever due to the action of a specific hæmolysin.

The resemblance between the action of specific hæmolytic serum and Black-water Fever is very striking.

It is easy to understand that in a crudely prepared serum (containing all the elements of the blood) wherein especially powerful agglutinins existed, the prominence of the erythrocatalytic process might be more accentuated than in Black-water Fever.

But though lysæmia is the characteristic feature of Black-water Fever our observations show that both processes of blood destruction, which seem characteristic of the action of blood destroying toxins acting in different ways, are concerned in Black-water Fever.

Parasitic, osmotic and chemical actions we have excluded as causes and it seems to us therefore most probable that Black-water Fever is due to some specific hæmolysin arising within the body as a result of the conditions we have pictured in the earlier parts of this memoir.

Part IV.

Nature of the Hæmolysin in Black-water Fever.

Chapter I.

Hæmolysins.

We have previously referred to sera which are able to destroy red cells of a particular animal both *in vitro* and *in vivo*.

It is necessary before going further to study more closely these specific hæmolytic bodies. Bordet (85) was the first to show that the serum of guinea-pigs, after they had been injected several times with 3 c.c. to 5 c.c. of defibrinated rabbit's blood, acquired the property of dissolving rapidly and intensely, in a test tube, the red cells of a rabbit; whereas such action by normal guinea pig serum is either absent or very feeble. This acquired property Bordet showed to be a specific one, but based upon a principle of general application. An animal of species A injected with the red cells of an animal of species B developes in its blood a greatly increased or entirely new solvent power for red cells of species B.

The study of this specific hæmolytic action has since gone on hand in hand with that of the bacteriolysins and other phenomena connected with immunity.

Bordet discovered that heating the hæmolytic serum to 55° Cent. for half an hour destroyed its solvent action; but that the addition of normal serum, itself devoid of hæmolytic power, restored this lost property.

Since then there has grown up a large body of facts relating to the "immune body," "sensibilisatrice" or "amboceptor," the thermostable body in hæmolytic sera and the "cytase," "alexine" or "complement" the thermolabile substance, which in combination with the immune body or amboceptor forms the complete and active hæmolysin.

In a literature so extensive as that relating to hæmolysis it is undesirable to follow the increase in our knowledge step by step. But it is necessary, in order that our own observations may be more easily followed, to give the chief facts known regarding the action of hæmolysins. The principal fact, the existence of two separate substances combining to form the active hæmolysin, has already been noted. According to Ehrlich's theory, which in its main propositions is now generally accepted, a hæmolysin consisting of an amboceptor *plus* a suitable complement can only act upon red cells which possess "side-chains" or "receptors" which fit the "haptophore group" or cell combining affinity of the amboceptor. We have thus to deal with three essential factors in hæmolysis by specific hæmolysins.

Receptors.

These according to Ehrlich's theory are side-chains in the central protoplasmic molecule of a cell, capable of combining with other free or external groups or receptors. It is upon these combining affinities that the cell is dependent for its normal metabolism; but their existence also exposes it to the toxic effects we are discussing. The receptors of one species of animal are foreign matter in the body of another species; but, provided the cells of the animal into which foreign blood has been injected possess side-chains or receptors capable of combining with those of the injected blood cells, combination at once takes place. But when the combination between the cell receptors of the injected animal and those of the foreign blood cells has taken place, the former cells are stimulated to produce new receptors to take the place of those already occupied.

These receptors are generally produced in excess and may be thrown off becoming free in the blood, and thus giving rise to an anti-body, specific for cells similar to those that stimulated its production. This anti-body possesses the power of combining with such foreign cells both *in vitro* and when injected *in vivo;* and if the necessary complement is present their combination will result in hæmolysis.

It is equally evident that upon the presence of suitable receptors will depend the effect of the injection of such specific toxins as the hæmolysins. We have seen that the injection into an animal of foreign blood cells, with their receptors, will cause the cells of the animal injected to produce and throw off an excess of receptors capable of combining with cells similar to those injected. These free receptors are amboceptors specific for similar foreign bloods, which necessarily possess receptors with which they can combine. If then such free amboceptors be injected in turn into an animal whose blood cells possess the suitable receptors, combination will ensue under certain conditions we shall further indicate.

If, on the contrary, the blood cells of an animal into which such an amboceptor is injected, possess no receptors with which it can combine, combination cannot occur and no reaction will ensue.

It will be necessary to refer more fully to the subject of receptors later on.

Amboceptors.

These, as we have already seen, are the free cell receptors that have been cast off in response to stimulation by foreign receptors. They possess in general certain properties which it is necessary to study—

(1) The power of combining with cells by means of a cytophile affinity.

(2) The power of combining with complement by means of their complementophile affinity.

Usually their cytophile affinity is stronger than their complementophile affinity, for they can combine with cells in the cold at a temperature of 0° Cent. at which temperature they will not combine with complement.

Amboceptors are as we have seen specific, they can enter into combination with only one kind of cell, that is cells possessing like receptors. But amboceptor alone, although it may enter into combination, cannot effect any definite change in the cell to which it becomes bound by its cytophile affinity. Its function then appears to be the linking up of a cell to complement or cytase, in such a way that this latter body which has toxic or ferment-like properties, can act and destroy the cell to which the amboceptor has united it.

We have seen therefore that amboceptors are not in themselves toxic, because they possess no "zymotoxic group," and cells plus amboceptor remain apparently undamaged and capable of performing all their normal functions.

We have also seen that amboceptor possesses such a strong affinity for cells with suitable receptors that it will enter into combination with them at exceedingly low temperatures.

Combination of red cells and amboceptor in vitro.—(1) To a suspension of red cells in ·8 per cent. salt solution, specific hæmolytic serum previously heated for ½ hour at 55° Cent. is added and the mixture is allowed to stand for a time. The red cells remain apparently unaltered, but on the addition of a little normal serum, which can be shown to have no action upon similar red cells without treatment, rapid hæmolysis ensues.

(2) To a suspension of red cells, unheated specific serum, capable of producing hæmolysis, is added and the mixture is maintained at a temperature of 0° Cent. for a time. No hæmolysis occurs. The mixture is then centrifuged and the red cells separated from the supernatant liquid. The red cells are apparently unaltered, but, on the addition of a little normal serum, speedy hæmolysis takes place. If the mixture of hæmolytic serum with red cells has been made in suitable amounts, the separated supernatant liquid will show, on further examination, that all the amboceptor has combined and been removed with the red cells, but that free complement still remains, its combination with the amboceptor having been prevented by the low temperature, at which therefore no hæmolysis occurred.

By a series of experiments such as these Ehrlich was able to show that red cells possess the power of combining with far more specific amboceptor than is sufficient to cause hæmolysis in the presence of complement. The amount taken up by them varies in different cases, but may be even one hundred times

in excess of that required in combination with complement for their complete destruction.

2. *Combination in the living body.*—In the absence of suitable complement it is theoretically possible, though such a condition has not, so far as we are aware, been described, for cells such as the red corpuscles to become laden with amboceptor in the body, such a combination producing no hæmolysis.

But a phenomenon of this nature has been recorded by Metchnikoff (87) in the case of guinea pigs inoculated with guinea pig spermatozoa. As the result of this inoculation, spermotoxin is formed in the blood serum; and this is shown to be toxic to the spermatozoa of the injected animal as well as to those of other guinea pigs. Although living and active spermatozoa may be seen in the epididymis and testes of the injected animal, they are no longer normal, for if placed in normal guinea pig serum, in which healthy guinea pig spermatozoa will live for several hours, they are immediately destroyed.

Thus although to all appearance normal, they have in reality become laden with immune body (amboceptor) which exposes them to rapid destruction in the presence of normal serum containing complement.

Complement.

Complement is, as we have already seen, a constituent of normal serum. In the presence of immune body (amboceptor) it can act upon various cells causing bacteriolysis, hæmolysis, etc. A number of different conceptions as to its exact nature have been put forward from time to time, some of which it will be necessary to discuss in greater detail later, when we are considering the treatment of Black-water Fever. But here we need only note that in its power of entering into combination with amboceptor it displays far less specificity than does amboceptor in combining with cells. Thus many different normal sera will provide complement that will combine with one specific amboceptor.

Although complement can be demonstrated in most sera, its existence in the plasma is a matter of discussion. Metchnikoff believes it to be derived from the dissolution of leucocytes, and to be a constituent of living cells, not of the plasma.

Leaving the nature of these cells an open question, we may say that this view seems to be generally admitted.

Absorption of Complement.

Complement can be absorbed *in vitro*, either by specific combination with amboceptor as in hæmolysis or bacteriolysis, or by the addition of a variety of substances; thus by digesting a serum with yeast cells, its complement may be entirely removed.

In specific absorption a fitting amboceptor must be present; and use is made of this reaction in testing for the presence of an amboceptor fitted to act against any given organism. In certain diseases absence of complement in the serum has been observed. Examples of this condition are syphilis and trypanosomiasis.

Hæmolysis in vitro and in vivo.

Hæmolysis *in vitro* has been the subject of much study, and has led to clear conceptions regarding the mechanism of the reaction under artificial conditions; but the factors which regulate hæmolysis within the body are still very obscure.

There is often a notable want of relation between action *in vitro* and action *in vivo*. Celli, Casagrandi and Craducci (77), while investigating experimental hæmoglobinuria, found that a serum, possessing no action *in vitro*, may on injection into an animal cause intense blood destruction, hæmoglobinuria and death. Lamb (72) notes that daboia venom is less hæmolytic *in vitro* than cobra venom, but more so *in vivo*.

At Major Lamb's suggestion we have tried *in vivo* the action of cobra-lecithid. This substance has all the components of a complete hæmolysin (amboceptor *plus* complement) and is *in vitro* strongly hæmolytic to rabbit's red cells.

But when injected into rabbits in doses of from ·01 to ·3 gramme it causes no observable blood destruction and does not produce the raising in the isotonic point of the corpuscles observed after the injection of daboia venom. Another illustration of want of relation between action *in vitro* and *in vivo* is shown by the injection into rabbits of a serum strongly hæmolytic *in vitro*. This serum we produced by immunising a calf against rabbit's blood.

Injected into rabbits in doses of ·15 c.c. it appeared to have no action. Possibly the formation of anti-complements produced co-incidently with the amboceptor during the process of immunising the calf with defibrinated rabbit's blood may have prevented action in this case.

In the case of the cobra-lecithid it appears possible that its apparent want of action *in vivo* may be due to its absorption by cells other than red cells possessing perhaps greater affinity and thus switching off the hæmolysin and preventing hæmolysis.*

The injection of a specific serum is, except in very large doses, not immediately followed by symptoms of blood destruction.

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* Arrhenius states that "normal rabbit serum is itself a little antitoxic to cobra-lecithid"; a property which it loses on heating for 30 minutes at 64° C.

(Immunochemistry by S. Arrhenius, page 213: New York, Macmillan Company, 1907.)

The following are observations of Celli, Casagrande, and Carduc.

Dog of six kilogrammes.

25th August.—Injected 5 c.m.c. of serum.
26th August.—Dog remains well.
27th August.—Injected another 5 c.m.c. of serum.
28th August.—Sick, pallor, etc.

Dog of 5·5 kilogrammes.

25th August.—Injection of 1 c.m.c. of serum under conjunctiva.
29th August.—Injection of 1 c.m.c. of serum.
1st September.—Death.

Our own experiments show:—

Dog I.

6 p.m., 10th February 1908.—Received 4 c.c. goat's serum.
Morning, 11th February 1908.—No distinct pallor of tongue.
8 a.m., 11th February 1908.—Received 2·5 goat's serum.
8 a.m., 12th February 1908.—Tongue pallid, conjunctiva pale.

Dog IV.

10 a.m., 2nd March 1908.—Received 15 c.c. goat's serum.
1 p.m., 2nd March 1908.—Dog shivering. Anæmia present.
5 p.m., 2nd March 1908.—Tissues pale, blood watery, etc.

Dog VI.

10 a.m., 2nd March 1908.—Received 3 c.c. goat's serum.
10 a.m., 3rd March 1908.—Passing hæmoglobinous urine.

Dog VII.

10 a.m., 2nd March 1908.—Received 1 c.c. goat's serum.
11 a.m., 5th March 1908.—Dog has been gradually getting more and more seedy.
Progressive anæmia now with weakness of hind legs as in piroplasma.

Dog VIII.

11 a.m., 5th March 1908.—Received 3 c.c. goat's serum.
4-30 p.m., 5th March 1908.—Isotonic point at ·83 per cent. salt.
8 a.m., 6th March 1908.—Isotonic point at 1 per cent. salt.

Dog IX.

11 a.m., 6th March 1908.—Received 6 c.c. goat's serum.
5 p.m., 6th March 1908.—Dog shows no symptoms.
10-30 a.m., 8th March 1908.—Dog [quiet, anæmia] marked but not profound, received 1·5 c.c. goat's serum.
1-30 p.m., 8th March 1908.—Pallor increased, dog profoundly ill.

Dog X.

5 p.m., 13th March 1908.—Received 3 c.c. goat's serum.
10 a.m., 14th March 1908.—No noticeable anæmia.
15th March 1908.—Marked pallor of tongue and a considerable amoun t of anæmia.

Dog XI.

5 p.m., 13th March 1908.—Received 6 c.c. goat's serum.
10 a.m., 14th March 1908.—Some pallor of tongue and some anæmia.
1 p.m., 14th March 1908.—Hæmoglobinuria.

Dog XII.

5 p.m., 13th March 1908.—Received 3 c.c. goat's serum and 10 c.c. normal goat's serum.
10 a.m., 4th March 1908.—Moderate anæmia.
4 p.m., 14th March 1908.—Extremities cold.
5 p.m., 15th March 1908.—Received 3 c.c. goat's serum.
16th March 1908.—Blood very anæmic, dog very seedy.

There is thus a variable period, during which the action of the poison is not apparent or slight, followed many hours or even days later by intense blood destruction.

To bring the facts relating to hæmolysis in the living body into harmony with the results of hæmolysis *in vitro* is not easy. We have seen in Part III that the injection of a hæmolytic serum does not necessarily lead to solution of the red cells but only to their engulfment. Whether engulfment takes place before there has been time for solution or whether the hæmolysin acts at all under these circumstances we do not know.

Barrat (88) has suggested that red cell phagocytosis is often stimulated as a result of the action of substances of the nature of opsonins often present in immune sera.

We think that the changes are to a certain extent due to agglutinins or possibly other bodies which accompany specific sera such as we are dealing with. It is however the conditions which give rise to the lysæmic form of blood destruction, the characteristic feature of Black-water Fever and paroxysmal hæmoglobinuria that more especially demand our attention.

What determines this condition we can as yet only surmise. The most important consideration as a basis for further work is the possible concerning of complement in the process.

In the absence of complement from the plasma, whether as a result of non-production (Metchnikoff we have seen denies that it exists free in the plasma) or of absorption, amboceptor, specific for the red blood cells if produced within the body, though it might combine with red cells could not bring about their extra-cellular solution.

If now under certain circumstances complement is suddenly produced and set free in the plasma whether within an organ or the general circulation, it will at once, combine with the amboceptor laden red cells, producing their rapid dissolution.

Whether such a combination of amboceptor with the red cell ever occurs or indeed what exactly happens in the case of a hæmolysin in the body we shall see to be an extremely difficult matter to determine.

The condition which we have turned extra-vascular lysæmia is also of extreme interest.

We have already seen that the condition generally described as hæmoglobinæmia does not mean the presence of hæmoglobin dissolved in the plasma during life. None the less the auto-solution of hæmoglobin in the serum during clotting of the blood may be of great significance. It is essentially the hæmolysis of a certain number of corpuscles consequent upon the act of coagulation; and this process we know to be associated with the formation of complement, a substance whose existence in the circulating blood is problematical.

If we conceive then of the liberation of complement in the presence of amboceptor-bound red cells within the body there might result lysæmia or true hæmoglobinæmia, but in the absence of complement from the plasma we may have the state of affairs giving rise to extra-vascular lysæmia.

Some support of this view is given by certain clinical facts. It has been frequently noted that in paroxysmal hæmoglobinuria the so-called hæmoglobinæmia was present when no attack was in progress. Bensuade (63) describes one case in which the serum was more coloured between than during the attacks, an observation which suggests that the red colouration of the serum is due to a condition characteristic of the blood in this disease, rather than to the liberation of hæmoglobin responsible for hæmoglobinuria.

In syphilis Justus (79) has shown that hæmoglobinæmia can be caused by producing stagnation of the blood in the vessels; and that blood removed from the median basilic vein after lightly constricting the arm for a few minutes may yield a reddish serum.

Chapter II.

The Demonstration of Autolysins.

The demonstration of isolysins is not fraught with any particular difficulties. It is essential only that a fitting complement and certain other conditions be present, and that there should be no anti-hæmolysin present. In the demonstration of autolysins on the other hand the greatest difficulty is encountered.

In the investigation of certain diseases in which hæmolytic action appears to be concerned, many observations have been made regarding the power supposed to be exerted upon normal red corpuscles by the pathological serum. Evidence of hæmolytic action has been considered an indication of the presence of the hæmotoxins which have initiated the blood changes in the patient.

Ehrlich (78) endeavouring to gain some insight into the nature of hæmolytic bodies which may arise within the body as a result of morbid processes, injected certain goats with large quantities of the blood of other goats. As a result he was able to produce hæmolysins within the bodies of the inoculated goats, but these hæmolysins had no action upon the corpuscles of the goats in whose bodies they arose; they were isolysins therefore and not autolysins.

From these experiments Ehrlich concludes that the demonstration that the serum of a patient possesses the power to dissolve the red cells of another person, does not prove that this action has had any relation in the patient to the occurrence of anæmia or any blood destruction. On the contrary it only demonstrates the presence of what is probably only an isolysin.

It is clear then that if we are to ascertain the exact mechanism of the blood-destroying process in Black-water Fever, we must especially study autolysins.

But before approaching the subject of autolysins it will be advantageous to study conditions that have resulted from the introduction of artificially produced hæmolysins in the body, and to see by what means apart from the recognition of its effects we may be enabled to demonstrate the presence of a hæmolysin that has once been introduced and absorbed into the system.

The Demonstration of Hæmolysins artificially introduced in the body.

Celli, Casagrandi, and Carducci (77), using dogs injected with the serum of rabbits previously treated with dogs' blood were the first to attempt to re-isolate or demonstrate the presence of the hæmolysin under these conditions.

At the outset they determined that the serum of a hæmoglobinuric dog has no action either upon a healthy dog's corpuscles or on those of a hæmoglobinuric dog.

The serum of a hæmoglobinuric dog plus the spleen of a healthy dog plus the corpuscles of a healthy dog was also negative. But the serum of a hæmoglobinuric dog plus the spleen of a healthy dog plus the corpuscles of a hæmoglobinuric dog, gave marked hæmolysis.

Applying these facts to the demonstration of a malarial hæmolysin in man, these observers got no result.

Approaching the subject afresh we sought in our own experiments on dogs to demonstrate the presence of the hæmolysin that we had ourselves injected. The corpuscles of the dog are very fragile, and if left standing for a few hours they are apt even in the case of the normal animal to show a certain amount of laking. This we obviated by the use of the solution of salt and sugar recommended by Blasi (90).

The results of our experiments were as follows :—

(1) The serum of dogs suffering from intoxication and hæmoglobinuria was not hæmolytic to the washed corpuscles from a healthy dog.

There was therefore no evidence of free hæmolysin.

(2) The addition of normal goat serum to the above did not bring about hæmolysis.

Therefore the absence of hæmolysis was not due to lack of complement but to absence of amboceptor, for normal goat serum contained fitting complement for the goat derived amboceptor we were using.

For *a priori* reasons also it appears hardly conceivable that any amboceptor could remain free in the presence of red cells which possessed suitable receptors for its cytophile group or affinity.

(3) The washed corpuscles of dogs suffering from intoxication were left at 37° Cent. suspended in salt and sugar solution. No hæmolysis or agglutination took place.

(4) Washed corpuscles from the hepatic, portal, renal, and heart blood were left at 37° Cent. in sugar and salt solution. No hæmolysis or agglutination resulted.

The red cells in neither the peripheral nor the visceral circulation were bound with hæmolysin (amboceptor and complement) for had they been so bound they should have dissolved after a short time.

(5) Pulp scraped from the spleen and washed to remove free hæmoglobin, and then left in ·9 per cent. salt solution, showed marked hæmolysis, but when placed in the sugar and salt solution no

hæmolysis occurred. Some agglutination appeared to be present. It is doubtful if even the red cells collected in the spleen were on the point of hæmolysis such as occurs *in vitro*.

(6) Washed red blood cells from the portal circulation *plus* crushed liver substance from a treated dog, showed very marked agglutination but no hæmolysis.

Agglutination of red cells was frequently very marked in the blood from the hepatic veins. There was also a similar tendency observed in blood from the kidneys, but an agglutinating effect of the injected dog's serum upon normal dog's corpuscles was never obtained. Agglutinins were, however, readily demonstrated in the œdema fluid about the site of the injection.

The demonstration of an injected hæmolysin or even the presence of agglutinins is therefore fraught with great difficulty. Nevertheless in spite of the absence of hæmolytic phenomena it is to be noted that the red cells of the treated dog could be shown to be abnormal by their behaviour on examination as regards—

(1) Change in the isotonic point.

(2) Change in appearance and staining re-action.

(3) Appearance of agglutination.

The Demonstration of Autolysins in Chronic Malaria.

Innumerable attempts have been made to demonstrate the hæmolytic agent in malaria, but with results either quite negative or unconvincing.

Celli, Casagrandi, and Carducci and later Blasi (91) testing the action of the serum of malaria patients against normal corpuscles failed to find evidence of hæmolytic power.

Similarly Capogrossi (94) ascertaining the agglutinating power of malarial serum against healthy corpuscles came to the conclusion that the appearance of agglutination was not diagnostic of malaria.

More recently Casagrandi claims to have discovered a hæmolysin masked by an ante-hæmolysin in the blood of a case of malarial hæmoglobinuria.

Examining the blood of a number of persons suffering from malaria and malarial anæmia we found the serum to possess no action upon healthy corpuscles, producing neither hæmolysis nor agglutination.

It seemed to us also that such a possibility as the occurrence of free amboceptor circulating in the plasma was very remote ; for if both amboceptor and

complement were present they would at once combine with the red cells, and hæmolysis would result.

But the possibility that in malaria as in trypanosomiasis complement might be so far absorbed, and that thus failing the completion of the hæmolysin, amboceptor might be bound by the red cells suggested to us the idea of searching for the presence of amboceptor-bound red cells in malaria.

The observations described by Metchnikoff, previously referred to in which guinea-pig spermatozoa became bound with amboceptor within the living body in the absence of complement appeared to indicate the condition which we might possibly find in the red cells of malarial patients.

Ehrlich suggests the complements can disappear during certain pathological conditions in consequence perhaps of a more rapid destruction or a slower production

In the constant blood destruction of malaria the using up of complement appeared an extremely likely phenomenon. Under such circumstances the red cells might become bound with amboceptor though to all appearances normal.

If such a condition were present it would only be necessary to add to washed malarial corpuscles a little normal human serum, and hæmolysis would occur.

When we came to examine a number of malarial patients we found that as a rule their red cells exhibited a great susceptibility to the action of normal serum so much so that rapid hæmolysis took place in some cases when only 50 c. m. of serum from certain normal subjects was added to $\frac{1}{2}$ c.c. of a five per cent. suspension of their red cells in ·8 per cent. salt solution. This hæmolysis was also invariably associated with a marked agglutination.

A reference to the literature showed that a similar susceptibility had been noted in the case of the blood of anæmic subjects in Italy by Ascoli (89).

On further testing the action of the serum of the malarial subjects we found that it not only failed to produce any hæmolysis of healthy blood corpuscles, but even in very small quantities inhibited the action of powerfully acting normal serum upon susceptible red cells.

Observation on the Blood in Black-water Fever.

Our results with the blood of Black-water Fever cases have been as follows. Further details of the cases are given in the Appendix :—

Black-water Fever serum	+	Washed normal corpuscles.	=	No hæmolysis. No agglutination.
Black-Water Fever serum	+	Normal corpuscles and normal serum.	=	No hæmolysis. No agglutination.

That is neither alone, nor with the undoubted presence of complement, had the Black-water Fever serum any action on the normal corpuscles used. (Class A., *vide* next chapter.)

Washed Black-water Fever corpuscles.	+	Allowed to stand in ·9% salt at room temp.	=	No hæmolysis. No agglutination.
Washed Black-water Fever corpuscles.	+	Black-water Fever serum	=	No effect.

These cells had therefore neither been already acted upon by an active hæmolysin, nor did the patient's own serum have any action upon them.

Black-water Fever corpuscles	+	Black-water Fever serum and normal serum (complement).	=	No hæmolysis.
Black-water Fever corpuscles	+	Serum of malarial patient	=	No hæmolysis. No agglutination.
Black-water Fever serum	+	Washed corpuscles of malarial patient.	=	Marked agglutination.

Case XX—

Black-water Fever serum	+	Washed normal corpuscles, S. R. C.	=	No hæmolysis. No agglutination.
Black-water Fever serum	+	Washed normal corpuscles, C. A. B.	=	No hæmolysis. No agglutination.
Black-water Fever serum	+	Washed normal corpuscles and normal serum (complement).	=	No hæmolysis. No agglutination.
Black-water Fever serum	+	Washed Black-water Fever corpuscles and normal serum (complement).	=	No hæmolysis. No agglutination.
Washed Black-water Fever corpuscles.	+	Allow to stand in ·9 per cent. salt at room temperature.	=	No hæmolysis. No agglutination.
Black-water Fever serum	+	Washed corpuscles of malarial patient.	=	Slight hæmolysis. Marked agglutination.
Normal serum, S. R. C.	+	Washed corpuscles of malarial patient.	=	Marked agglutination.
Normal serum, C. A. B.	+	Washed corpuscles of malarial patient.	=	Marked agglutination.
Washed Black-water Fever corpuscles.	+	Black-water Fever serum and extract of dog's spleen.	=	Some hæmolysis.
Normal corpuscles	+	Normal serum and extract of dog's spleen.	=	Some hæmolysis.

Case XXII—

Washed Black-water Fever corpuscles.	+	Normal serum	=	Hæmolysis.
½ c.c. washed Black-water Fever corpuscles emulsion.	+	(50 c.m. Black-water Fever normal serum) at 37 for 15 min.)	=	No hæmolysis. Agglutination.
½ c.c. washed corpuscles native	+	150 c.m. Black-water Fever serum.	=	No hæmolysis. No agglutination.
½ c.c. washed corpuscles very anæmic native.	+	150 c.m. Black-water Fever serum.	=	Agglutination.

Case XXIV—

½ c.c. washed Black-water Fever corpuscles emulsions.	+	150 c.m. normal serum	=	Rapid hæmolysis.
½ c.c. washed Black-water Fever corpuscles emulsions.	+	(150 c.m. normal serum, 50 c.m. Black-water Fever serum (at 37 for 15 min.)	=	No hæmolysis. Agglutination.
Washed Black-water Fever corpuscles.	+	Allowed to stand in ·9 per cent. salt.	=	No hæmolysis.

Case XXV—

½ c.c. washed Black-water Fever corpuscles emulsions.	+	150 c.m. normal serum, S. R. C.	=	No hæmolysis. No agglutination.
Washed Black-water Fever corpuscles.	+	Undiluted normal serum, S. R. C.	=	No hæmolysis. No agglutination.

Case XXVI—

Washed Black-water Fever corpuscles.	+	Allowed to stand in ·9 per cent. salt room temperature.	=	No hæmolysis.
Black-water Fever corpuscles	+	Black-water Fever serum	=	No hæmolysis. No agglutination.
½ c.c. washed Black-water corpuscles emulsions.	+	150 c.m. normal serum	=	Complete hæmolysis.
½ c.c. washed Black-water Fever corpuscles emulsions.	+	150 c.m. normal serum, 50 c.m. Black-water Fever serum (at 37 for 15 min.)	=	Hæmolysis slightly retarded.
½ c.c. washed Black-water Fever corpuscles emulsions.	+	150 c.m. normal serum = 100 c.m. Black-water Fever serum (at 37 for 15 min.)	=	No immediate hæmolysis. Slight after some hours. Agglutination.

Case XXVII—

Washed Black-water Fever corpuscles.	+	Allowed to stand in ·9 per cent. salt at room temp.	=	No hæmolysis.
Washed normal corpuscles	+	Undiluted Black-water Fever serum.	—	No hæmolysis. No agglutination.

Washed normal corpuscles	+	Undiluted Black-water Fever serum (at 37 for 30 min.) centrifuged normal serum.	= { No hæmolysis. No agglutination.
½ c.c. washed Black-water Fever corpuscles emulsions.	+	150 c.m. normal serum	= { No hæmolysis. No agglutination.
Washed Black-water Fever corpuscles.	+	Undiluted normal serum	= No agglutination.
½ c.c. washed corpuscles, native (Class B). (*Vide* next chapter.)	+	300 c.m. Black-water Fever serum.	= { No hæmolysis. Marked agglutination.
½ c.c. washed corpuscles, native (Class B).	+	150 c.m. normal serum, S. R. C.	= Complete hæmolysis.
½ c.c. washed corpuscles, native (Class B).	+	50 c.m. normal serum 50 c.m. Black-water Fever serum (at 37 for 15 min.)	= { No hæmolysis. No hæmolysis.
½ c.c. washed corpuscles, native (Class B.)	+	150 c.m. normal serum 20 c.m. Black-water Fever serum (at 37 for 15 min.)	= Hæmolysis.

In this case there was therefore antibody, in spite of the fact that the blood was a modified Class A (*vide* next chapter).

These results were intelligible only after we had gained the further knowledge detailed in the next chapter.

CHAPTER III.

HUMAN ISOLYSINS.

When we found that the red cells of people anæmic from malarial infection were characterised by a marked susceptibility to hæmolysis on the addition of normal serum we at first considered that this observation pointed to a binding by them of amboceptor, which, on the addition of normal serum containing complement, resulted in their speedy hæmolysis.

We have since added to our observations with the result that our original idea has been shown to be untenable. But our observations have served to point to a condition we believe not generally recognised, and of which we can find no indication in the work of Ehrlich and others. Isolysins in man have generally been considered an occasional phenomenon due to some pathological condition of the person whose serum was isolytic. That the red cells against which such a serum was tested might be an equally important factor has not generally been taken into account.

If therefore in the serum of a Black-water Fever case we were to demonstrate isolysins it might be thought that we had shown a point of pathological importance.

Again we have seen that in malaria we appeared to have found red cells bound with immune body (amboceptor) since on the addition of normal serum they became rapidly hæmolysed.

But the real facts possess quite a different significance and make all work on pathological isolysins undertaken in ignorance of them, open to constant error.

And the same may be said of any attempt to demonstrate the presence of autolysins in disease.

The following experiments will make this clear.

I.—Experiments to shew susceptibility of washed blood corpuscles of Class B bloods to hæmolysis and agglutination by Class A serum and want of action either hæmolytic or agglutinant of Class B serum upon washed corpuscles of Class A or Class B blood.

SERIES I.

Case I.—Child ; jaundice and anæmia.

Child's washed corps.	+	Small quantity normal serum, S. R. C.	=	Marked agglutination.
Washed corps., S. R. C.	+	Small quantity child's serum.	=	*Nil.*

Washed corps. child in ·9 per cent. salt.		=	*Nil.*
Washed corps. child	+ Child's serum	=	*Nil.*

Case II.—Adult ; extreme anæmia (malaria).

1 c.c. emuls. corps.	+ 20 c.m. normal serum, S. R. C.	= Marked agglutination.
1 c.c. emuls. corps.	+ 120 c.m. normal serum, S. R. C.	= Rapid and complete hæmolysis.
1 c.c. emuls. corps.		= *Nil.*
1 c.c. emuls. normal corps., S. R. C.	+ 120 c.m. serum, No. 2	= *Nil.*

Case III.—Adult ; fever and enlarged spleen, no parasites.

½ c.c. emuls. corps.	+ 1 c.m. normal serum, S. R. C.	= Agglutination.
½ c.c. ,, ,,	+ 5 c.m. ,, ,,	= Trace of hæmolysis and agglutination.
½ c.c. ,, ,,	+ 20 c.m. ,, ,,	= ,, ,, ,, ,,
½ c.c. ,, ,,	+ 40 c.m. ,, ,,	= Considerable hæmolysis.
½ c.c. ,, ,,	+ 60 c.m. ,, ,,	= Nearly complete hæmolysis.
½ c.c. ,, ,,	+ 100 c.m. ,, ,,	= Complete hæmolysis in ½ hour.
½ c.c. ,, ,,	+ 1 c.m. normal serum, C. A. B.	= *Nil.*
½ c.c. ,, ,,	+ 5c m. ,, ,,	= Trace of hæmolysis.
½ c.c. ,, ,,	+ 20 c.m. ,, ,,	= Slight hæmolysis.
½ c.c. ,, ,,	+ 40 c.m. ,, ,,	= Marked hæmolysis.
½ c.c. ,, ,,	+ 60 c.m. ,, ,,	= ,, ,,
½ c.c. ,, normal corps.	+ 20 serum No. 3, S. R. C.	= *Nil.*
½ c.c. ,, normal corps. C. A. B.	+ 120 serum, Case 3	= *Nil.*

Case IV.—Boy ; Simple Tertian infection parasites average 1 per field.

½ c.c. emuls. corps.	+ 10 c.m. serum, S. R. C.	= Agglutination.
½ c.c. ,, ,,	+ 40 c.m. ,, ,,	= ,,
½ c.c. ,, ,,	+ 200 c.m. ,, ,,	= Almost complete hæmolysis.
½ c.c. corps., S. R. C.	+ 200 c.m. serum, Case 4	= *Nil.*

Case V.—Adult ; anæmic.

½ c.c. emuls. corps.	+ 150 serum, S.R.C.	= Hæmolysis.
½ c.c. ,, ,,	+ 150 ,, Case 3	= *Nil.*
½ c.c. ,, ,, Case 4	+ 20 ,, ,,	= *Nil.*
½ c.c. ,, ,, ,,	+ 100 ,, ,,	= *Nil.*
½ c.c. ,, ,, ,,	+ 250 ,, ,,	= *Nil.*
½ c.c. ,, ,, ,, 3	+ 150 c.m. ,, ,,	= *Nil.*

Case VI.—Adult; European visitor to the Duars.

½ c.c. emuls. corps,	Case 4	+	20 c.m. serum,	Case 6	=	Agglutination.
½ c.c.	„ „ „	+	100 c.m.	„ „	=	Marked hæmolysis.
½ c.c.	„ „ „ 6	+	20 c.m.	„ S.R.C.	=	*Nil.*
½ c.c.	„ „ „	+	100 c.m.	„ „	=	*Nil.*
½ c.c.	„ „ „	+	250 c.m.	„ „	=	*Nil.*

Case VII.—Adult; enlarged spleen, prisoner, Jalpaiguri jail.

½ c.c. emuls. corps.,	Case 7	+	150 c.m. serum,	S. R. C.	=	Slight hæmolysis; agglutination.
½ c.c.	„ „ „	+	150 c.m.	„ Case 8	=	Fair hæmolysis; agglutination.
½ c.c.	„ „ „	+	150 c.m.	„ „ 9	=	*Nil.*

Case VIII.—Adult; no spleen; anæmia; prisoner, Jalpaiguri jail.

½ c.c. emuls. corps.,	Case 8	+	150 c.m. serum,	S. R. C.	=	*Nil.*
½ c.c.	„ „ „	+	150 c.m.	„ Case 7	=	*Nil.*
½ c.c.	„ „ „	+	150 c.m.	„ „ 9	=	*Nil.*

Case IX.—Adult; slightly enlarged spleen; prisoner, Jalpaiguri jail.

½ c.c. emuls. corps.,	Case 9	+	150 c.m. serum,	S. R. C.	=	Complete hæmolysis.
½ c.c.	„ „ „	+	150 c.m.	„ Case 7	=	Slight „
½ c.c.	„ „ „	+	150 c.m.	„ „ 8	=	Complete „

Case X.—Adult; no spleen; has lived many years on an unhealthy garden in the Duars.

½ c.c. emuls. corps.		+	150 c.m. serum,	S.R.C.	=	Marked hæmolysis.
½ c.c.	„ „	+	100 c.m.	„ C.A.B.	=	„ „
½ c.c.	„ „	+	150 c.m.	„ Case 3	=	*Nil.*
½ c.c.	„ „	+	150 c.m.	„ „ 7	=	Marked hæmolysis.
½ c.c.	„ „	+	150 c.m.	„ „ 8	=	„ „
½ c.c.	„ „	+	150 c.m.	„ „ 9	=	*Nil.*
½ c.c.	„ „	+	150 c.m.	„ „ 11	=	Marked hæmolysis.
½ c.c.	„ „	+	150 c.m.	„ „ 12	=	*Nil.*

II.—Experiments to show the existence of an antibody in the serum of Class B preventing hæmolytic effect of Class A serum upon Class B corpuscle but not agglutination.

(*a*) Class B serum preventing the action of Class A serum against its own corpuscles.

½ c.c. emuls. corps.,	Case 10	+	100 c.m. serum,	S.R.C.	=	Nearly complete hæmolysis.
½ c.c.	„ „ „	10 +	100 c.m. serum and 50 c.m. serum No. 10		=	Trace of hæmolysis.

½ c.c. emuls. corps., Case 10 + { 100 c.m. serum, S.R.C. / 50 c.m. serum No. 10 / at 37 for 15 m. } = *Nil.*

½ c.c. „ „ „ 12 + 20 c.m. serum, S.R.C. = Agglutination.

½ c.c. „ „ „ + 50 c.m. „ „ = Fair hæmolysis; agglutination.

½ c.c. „ „ „ + 100 c.m. „ „ = Marked hæmolysis.

½ c.c. „ „ „ + 150 c.m. „ „ = „ „

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / 5 c.m. serum, Case 12 / at 37 for 15 m. } = Nearly complete hæmolysis.

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / 20 c.m. serum, Case 12 / at 37 for 15 m. } = *Nil* at first, but slight after standing some time; agglutination.

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / 50 c.m. serum, Case 12 / at 37 for 15 m. } = No hæmolysis; agglutination.

(*b*) Class B serum preventing the action of Class A serum against other Class B corpuscles.

½ c.c. emuls. corps, Case 10 + 150 c.m. serum, S.R.C. = Nearly complete hæmolysis.

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / 150 c.m. serum, Case 12 / at 37 for 15 m. } = No hæmolysis; agglutination.

½ c.c. „ „ „ + { 150 c.m. serum S.R.C. / 50 c.m. serum, Case 12 / at 37 for 15 m. } = „ „ „

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / 20 c.m. serum, Case 12 / at 37 for 15 m. } = Slight hæmolysis; agglutination.

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / 5 c.m. serum, Case 12 / at 37 for 15 m. } = Fair hæmolysis; agglutination.

III.—Experiments to show that the hæmolytic action of Class A serum upon Class B corpuscles is destroyed by heating to 55°.

½ c.c. emuls. corps., Case 10 + 150 c.m. serum, S.R.C. = Nearly complete hæmolysis.

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / heated to 55 for 36 m. } = *Nil.*

½ c.c. „ „ „ 3 + 150 c.m. serum, S.R.C. = Complete hæmolysis.

½ c.c. „ „ „ + { 150 c.m. serum, S.R.C. / heated to 55 for 20 m. } = *Nil.*

IV.—Experiments to ascertain whether the hæmolytic effect of Class A serum upon Class B corpuscles is due to the addition of complement.

Experiment I.

Serum of No. 18 series 2 (strong Class A) + corpuscles of C (susceptible) kept on ice for 1 hour. The supernatant fluid and corpuscles being then rapidly separated by centrifugaling.

Supernatant fluid	+	Corps C	= Trace of hæmolysis only.
Corps. separated by centrifugalisation.	+	Serum No. 23 series 2 (Class B).	= *Nil.*
Ditto	+	Serum No. 13 series 2 (Class A.)	= Marked hæmolysis.

Experiment II.

Serum S.R.C. (Class A) corpuscles No. 23 series 2 (suscept.) kept on ice for 1 hour Supernatant fluid rapidly separated.

Supernatant fluid	+	Corps. No. 23 series 2	= *Nil.*
Corpuscles	+	Serum No. 23 series 2	= *Nil.*

The amboceptor appears to have been removed by B corpuscles from the A serum.

Experiment III.

6 c.c. salt solution + ·6 c.m., S. R. C. serum placed on ice. When cooled to 0°, added excess of corpuscles No. 33 series 2 (susceptible). Left on ice for 1 hour and pipetted off clear fluid left by settlement of the corpuscles.

Control ·3 c.c. salt	+	·150 c.m. S. R. C. serum left on ice for 1 hour.	
Supernatant fluid	+	corps. No. 33 series 2	= *Nil.*
Control	+	„ „ 33 series 2	= Complete hæmolysis.
Inactivated S.R.C. serum	+	experimental fluid and corps. No. 33 series 2	= Distinct hæmolysis.

Complement appears to be still present after digestion at 0° with excess of susceptible cells.

These experiments are therefore in favour of the view that the hæmolytic effect is due to an isolysin in Class A bloods acting upon class B corpuscles.

V.—Experiments to show that there is no amboceptor in Class B serum capable of being bound by Class A corpuscles.

Experiment I.

Kept at 0°C for 2 hours corpuscles washed and left in Class A serum 37°C.

50 c.m. serum No. 10 series 1	+	S.R.C. corps. contained in ½ c.c. emulsion	= *Nil.*
50 c.m. „ „ „	+	S.R.C. corps. contained in ½ c.c. emulsion	= *Nil.*
50 c.m. „ „ „	+	S.R.C. corps. contained in 1 c.c. emulsion	= *Nil.*
50 c.m. „ „ „	+	S.R.C. corps. contained in 1½ c.c. emulsion	= *Nil.*

Experiment II.

Corps., S.R.C.	+	Pure serum anæmic coolie with susceptible corps. centifugalised and corps. washed.	=	*Nil.*
Washed treated corps.	+	150 c.m. serum, S.R.C.	=	No hæmolysi

VI.—Experiments to show that Antibody acts better against the isolytic serum than as a protective body to corpuscles.

½ c.c. emuls. corps. 10 series I 50 c.m. serum 12 series I.	+	150 c.m. serum, S.R.C.	= Fair hæmolysis.
½ c.c. emuls. corps. 10 series I	+	150 c.m. serum, S.R.C. 50 c.m. serum, No. 12 at 37 for 15 m.	= *Nil.*
½ c.c. emuls. corps. 12 series I 50 c.m. serum 12 series I	+	150 c.m. serum, S.R.C.	= Slight hæmolysis.
½ c.c. emuls. corps. 12 series I	+	150 c.m. serum, S.R.C. 50 c.m. ser. No. 12 at 37 for 15 m.	= *Nil.*
20 c.m. emuls. corps. anæmic coolie.	+	150 c.m. serum, S.R.C.	= Slight hæmolysis.
c.c. emuls. corps. anæmic coolie.	+	150 c.m. serum, S.R.C. 20 c.m. serum anæmic coolie.	= *Nil.*

VII.—Experiments to show that Antibody is not destroyed by heating to 55° C.

½ c.c. emuls. corps. 10 series I	+	150 c.m., S.R.C. serum	= Nearly complete hæmolysis.
c.c. „ „ „ „ I	+	150 c.m. serum, S.R.C. 100 c.m. serum No. 12 inactivated at 37 for 15 m.	= *Nil.*

VIII.—To show that there is a binding by susceptible corpuscles of substances in Class A serum when these are too small in amount to produce hæmolysis.

·4 c.c. of a 4 per cent. emuls.	+	150 c.m. serum, Class A	= No hæmolysis; agglutination.

Allowed to remain at 37° for 15 m. centrifuged and corpuscles shaken up in ·4 c.c. fresh ·9 per cent. salt.

·4 c.c. of a 4 per cent. emuls. treated corpuscles	+	150 c.m. serum, Class A	= Hæmolysis.
Control	+	150 c.m. serum, Class A.	= No hæmolysis.

IX.—To show that the amount of hæmolysis is dependent on:—

(a) *dilution of the serum;*

(b) *amount of corpuscles.*

·4 c.c. of a 2 per cent. emuls. corps.	+	150 c.m. serum Class A	= Fair hæmolysis.
·4 c.c. „ 2 „ „ „	+	300 „ „ „ „	= Rapid and nearly complete hæmolysis.
·4 c.c. „ 4 „ „ „	+	150 „ „ „ „	= Agglutination only.
·1 c.c. „ 2 „ „ „	+	150 „ „ „ „	= Complete hæmolysis.
·1 c.c. „ 2 „ „ „ *plus* ·4 c.c. „ ·9 „ salt.	+	150 „ „ „ „	= Nearly complete but delayed.

X.—*Experiments to show uniformity of behaviour of Class A and Class B bloods to one another.*

SERIES 2.

European soldiers six weeks in India.

150 c.m. serum.	EMULSION OF WASHED CORPUSCLES IN '9 PER CENT. SALT.												
	Soldier 1	Soldier 2	Soldier 3	Soldier 4	Soldier 5	Soldier 6	Soldier 7	Soldier 8	Soldier 9	Soldier 10	Soldier 11	Soldier 12	S.R.C.
S.R.C.	*Nil*	H	h	*Nil*	h	*Nil*	H	H	*Nil*	*Nil*	*N*	*Nil*	*Nil*
Soldier 1	...	H	...	...	...	*Nil*	...	...	...	...	...	...	...
„ 2	...	...	...	...	..	*Nil*	...	...	...	...	...	...	...
„ 3	...	...	...	...	..	...	...	...	...	...	...	...	...
„ 4	...	...	...	...	...	...	...	...	...	...	...	...	...
„ 5	...	...	...	...	...	..	...	...	...	...	...	...	...
„ 6	*Nil*	...	..	...	...	...	...	...	...	...	...	...	...
„ 7	*Nil*	...	..	...	...	...	...	...	...	...	...	...	...
„ 8	*Nil*	...	...	...	...	...	...	...	...	..	..	...	...
„ 9	...	...	...	...	...	...	...	...	...	...	...	...	..
„ 10	...	..	...	...	h	...	...	...	...	...	..	...	...
„ 11	...	...	...	...	h	...	...	...	...	...	...	...	...
„ 12	...	...	...	..	h	*Nil*	...	...	...	...	...	...	...

Action of the above sera upon washed corpuscles of "C" a Class B native blood.

—	1	2	3	4	5	6	7	8	9	10	11	12	S.R.C
150 c.m. serum · ½ c.c. emuls. corps. "C."	H	*Nil*	h	h	*Nil*	*Nil*	*Nil*	*Nil*	H	H	H	H	H
Class .	A	B	B	A	B	A	B	B	A	A	A	A	A

150 c.m. serum.	½ C.C. EMULSION OF WASHED CORPUSCLES IN ·9 PER CENT. SALT. BLOODS 13 TO 24.												
	Soldier 13	Soldier 14	Soldier 15	Soldier 16	Soldier 17	Soldier 18	Soldier 19	Soldier 20	Soldier 21	Soldier 22	Soldier 23	Soldier 24	C.A.B.
S.R.C.	h	h	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	h	H	h	*Nil*
Soldier 13	...	...	*Nil*	*Nil*	...	...	...	...	...	...	...	...	...
„ 14	...	...	...	*Nil*	...	...	...	...	*Nil*	...	...	...	...
„ 15	h	...	...	...	...	...	...	...	*Ni*	...	...	...	...
„ 16	...	...	*Nil*	...	...	...	...	...	...	...	h	*Nil*	...
„ 17	...	...	...	...	...	..	...	*Nil*	...	H	H	H	...
„ 18	H	H	*Nil*	*Nil*	...	...	...	...	...	...	H	...	...
„ 19	...	...	...	...	...	...	...	...	...	...	H	...	*Nil*
„ 20	...	...	...	...	...	...	...	...	...	H	...	...	...
„ 21	H	...	*Nil*	*Nil*	...	...	...	...	...	...	...	...	*Nil*
„ 22	...	...	...	...	..	...	...	...	...	...	...	...	...
„ 23	...	...	...	..	...	...	...	...	...	...	...	...	...
„ 24	*Nil*	...	...	...	...	...	...	*Nil*	...	...	...	...	...

Action of sera upon washed corpuscles of "C" a Class B, native blood.

—	13	14	15	16	17	18	19	20	21	22	23	24	C.A.B.
150 c.m. ser. ½ c.c. emul. corps. "C."	h	h	H	H	H	H	H	h	H	h	*Nil*	*Nil*	H
Class .	B	B	A	A	A	A	IA	A	IA	B	B	B	A

		150 c.m. serum S.R.C.	150 c.m. serum C.A.B.	—		½ c.c. emul. corps. C.
½ c.c. emul. corps. of Soldier	25	H	...	150 c.m. serum of	25	*Nil*
" " " "	26	*Nil*	...	" "	26	*Nil*
" " " "	27	*Nil*	...	" "	27	*Nil*
" " " "	28	...	h	" "	28	*Nil*
" " " "	29	*Nil*	*Nil*	" "	29	h
" " " "	30	*Nil*	...	" "	30	h
" " " "	31	*Nil*	...	" "	31	H
" " " "	32	...	*Nil*	" "	32	h
" " " "	33	H	H	" "	33	*Nil*
" " " "	34	...	*Nil*	" "	34	h
" " " "	35	H	...	" "	35	h
" " " "	36	*Nil*	...	" "	36	*Nil*
" " " "	37	H	...	" "	37	*Nil*
" " " "	38	H	...	" "	38	*Nil*
" " " "	39	*Nil*	...	" "	39	*Nil*
" " " "	40	H	...	" "	40	*Nil*
" " " "	41	h	...	" "	41	*Nil*
" " " "	42	H	...	" "	42	*Nil*
" " " "	43	*Nil*	...	" "	43	...
" " " "	44	*Nil*	...	" "	44	...
" " " "	45	*Nil*	...	" "	45	...
" " " "	46	*Nil*	...	" "	46	...
" " " "	47	H	...	" "	47	...
" " " "	48	H	...	" "	48	...
" " " "	49	H	...	" "	49	...

SERIES 3.

European Sailors.

150 c.m. serum.	½ c.c. emulsion washed corpuscles.					
	Sailor 1	Sailor 2	Sailor 3	Sailor 4	Sailor 5	Sailor 6
S. R. C. . . .	*Nil*	h	*Nil*	H	h	h
C. A. B. . . .	*Nil*	h	*Nil*	H	h	
No. 10, series 4 .	*Nil*	H	*Nil*	H	...	...
No. 12, series 4 .	...	...	...	...	H	...
I . .	...	...	...	h	...	...
No. 2, series 4 .	...	...	...	h	...	...
No. 4, series 4 . .	...	...	...	H	...	...
No. 9, series 4 .	...	...	...	h	...	...

Action of the Sera upon washed corpuscles of "C."

150 c m. serum.	1	2	3	4	5	
½ c.c. emuls. corps. "C."	h	*Nil*	H	H	*Nil*	
Class . . .	A	B	A	?	B	

Details of Blood No. 4—

½ c.c. emuls. corps.	4 +	150 serum 1 50 serum 4 at 37 for 15 m.	= *Nil.*
½ c.c. ,, ,,	4 +	150 serum 1	= Marked hæmolysis.
½ c.c. ,, ,,	5 +	150 serum 1 50 serum 4 at 37 for 15 m.	= *Nil.*
½ c.c. ,, ,,	5 +	150 serum 1	= Slight hæmolysis.
½ c.c. ,, ,,	7 +	150 serum 4	= Fair hæmolysis.

Blood 4 is susceptible to A sera and has antibody against these, but it is also isolitic to certain B corpuscles.

Series 4.

Long term prisoners in Calcutta Presidency Jail.

½ c.c. emuls. corps.	150 c.m. serum of					150 c.m. serum.	½ c.c. emuls. corps. C.
	S.R.C.	C.A.B.	No. 2 series 3.	Prisoner 3.	Prisoner 20.		
Prisoner 1 .	H	...	...	...	...	1	*Nil*
„ 2 .	*Nil*	..	...	...	...	2	H
„ 3 .	*Nil*	...	...	...	...	3	H
„ 4 .	*Nil*	...	h	...	...	4	H
„ 5 .	H	...	h	...	...	5	*Nil*
„ 6 .	H	...	*Nil*	...	...	6	*Nil*
„ 7 .	H	...	h	...	...	7	*Nil*
„ 8 .	*Nil*	..	*Nil*	...	...	8	H
„ 9 .	...	*Nil*	*Nil*	*Nil*	...	9	H
„ 10 .	...	*Nil*	...	*Nil*	...	10	H
„ 11 .	...	H	...	H	...	11	*Nil*
„ 12 .	...	...	...	*Nil*	...	12	H
„ 13 .	h	...	...	...	...	13	*Nil*
„ 14 .	H	*Nil*	...	...	...	14	*Nil*
„ 15 .	H	h	...	...	...	15	*Nil*
„ 16 .	h	*Nil*	...	...	...	16	*Nil*
„ 17 .	H	h	...	...	...	17	*Nil*
„ 18 .	H	h	...	...	...	18	*Nil*
„ 19 .	...	H	...	...	H	19	*Nil*
„ 20 .	*Nil*	*Nil*	...	...	...	20	H
„ 21 .	H	*Nil*	...	...	...	21	*Nil*
„ 22 .	*Nil*	*Nil*	...	...	...	22	H
„ 23 .	*Nil*	*Nil*	...	...	...	23	*Nil*

SERIES 4—*contd.*

½ c.c. emuls. corps.	150 c.m. serum of						150 c.m. serum.	½ c.c. emuls. corps. C.
	S.R.C.	C.A.B.	Prisoner 24.	Prisoner 27.	Prisoner 29.	Prisoner 30.		
Prisoner 24 . .	h	...	...	*Nil*	*Nil*	*Nil*	24	h
„ 25 . .	h	*Nil*	...	...	...	...	25	*Nil.*
„ 26 . .	h	*Nil*	...	...	...	...	26	*Nil.*
„ 27 . .	H	H	h	...	...	...	27	*Nil.*
„ 28 . .	H	*Nil*	...	...	...	...	28	*Nil.*
„ 29 . .	H	h	*Nil*	...	...	...	29	h
„ 30 . .	H	h	h	...	...	...	30	*Nil.*
„ 31 . .	h	...	...	...	...	...	31	*Nil.*
„ 32 . .	h	...	...	...	...	...	32	*Nil.*
„ 33 . .	H	...	...	...	...	...	33	*Nil.*
„ 34 . .	H	...	...	...	...	...	34	*Nil.*
„ 35 . .	h	...	...	...	...	...	35	h

XI.—Condition of Blood in Residents of the Duars.

SERIES 5.

½ c.c. emuls. corps.	150 c.m. serum S.R.C.	150 c.m. serum 1.	150 c.m. serum C.A.B.	Antibody.
1. Babu, 24 years' resident .	*Nil*	...	...	
2. Babu, 2 years' resident .	H	...	...	
3. Babu, 1 year's resident .	H	H	Agg.	150 c.m. serum, S.R.C. + 20 c.m. 3 at 37°C. for 15 m. } + corps. 3 *Nil.*
4. European, 5 years' resident	H	...	Agg.	
5. Babu, 6 months' resident .	H	...	Agg.	
6. Babu, many years' resident	H	...	...	150 c.m. serum, S.R.C. + 50 c.m. serum C.A.B. at 37°C. for 15 m. } + co ps. 3 =H.
7. European, 25 years' resident	*Nil*	...	...	150 c.m. serum 7 + corps. 8=No H. 150 c.m. serum, S.R.C. 150 c.m. serum 7 at 57°C. for 15 m. } + corps. 8= H.
8. European, 13 years' resident 3 attacks of Black-water Fever.	H	...	...	
9. European, new arrival .	*Nil*	*Nil*	*Nil*	

SERIES 6.

½ c.c. emuls. corps.	150 c.m. serum, S.R.C.	150 c.m. serum 3.	150 c.m. serum 7.	Antibody.
1. Babu, 26 years' resident .	H	...	...	
2. Babu, 20 years' resident .	H	...	...	
3. Babu, 4 years' resident .	H	...	...	
4. Babu, from birth . .	*Nil*	...	...	150 serum 7 / 150 c.m. serum 4 at 37°C. for 15 m. } +corps. 6=H.
5. European, 6 years' resident	H	...	...	
6. European, 10 months' resident.	H	*Nil*	H	150 c.m. serum 7 / 50 (serum 6) } +corps. 6=H. 150 c.m. serum 7 / 150 c.m. serum 6 at 37°C. for 15 m. } +corps. 6=h.
7. European, 13 years' resident, Black-water Fever 1 year ago, taking 5 grs. quinine daily for last 6 months.	*Nil*	...	...	
8. European, 12 years' resident	*Nil*	...	...	
9. European, 4 months' resident.	H	...	...	200 c.m. serum, S.R.C. / 50 c.m. serum 9 at 37 for 15 m. } +corps. *Nil.* 9=
10. European, 1 year resident	H	...	...	200 c.m. serum, S.R.C. / 50 c.m. serum 10 at 37 for 15 m. } +corps. *Nil.* 9= Ditto {+corps. 5 series 7.} =H.
11. European, 5 months' resident. Contracted severe malaria 1st fortnight. Anæmic.	H	...	...	150 c.m. serum, S. / 50 c.m. serum 11 at 73 for 15 m. } +corps. H. 11=
12. European, 18 months after return from home. Five months at Neora Ndy. Seedy, having fever.	H	...	...	150 c.m. serum, S.R. C. / 50 c.m. serum 1 at 47 for 1 5m. } +corps. H. Some delay. 12= 150 c.m. serum, S.R.C. / 100 c.m. serum 12 at 37 for 15 m. } +corps. H. Some delay. 12=
13. Babu, 5 years' resident.' Case 20. Four months after attack.	H	...	...	150 c.m. serum, S.R.C. / 20 c.m serum 13 at 37 for 15 m. } +corps. H. 13= 150 c.m. serum, S.R.C. / 100 c.m. serum 13 at 37 for 15 m. } +corps. *Nil.* 13=
14. European, old resident	H	...	...	150 c.m. serum, S.R.C. / 20 c.m. serum 14 at 37 for 15 m. } +corps H. 14= 150 c.m. serum, S.P.C. / 50 c.m. serum 14 at 37 for 15 m. } +corps. *Nil.* 14=

SERIES 7.

XII.—Condition of Blood in Native children.

½ c.c. emuls. corps.	150 c.m. serum S.R.C.	Antibody.
1. Child, 1 month's resident. Fever S. T. parasites. No enlargement spleen.	H	.....
2. Child, 3 months' resident. Fever. Q No enlargement spleen.	*Nil.*	.....
3. Child, 1 month's resident. Spleen palp.	H	.....
4. Child, 1 month's resident. Enlargement spleen.	H	.....
5. Child, 3 months' resident. Enlargement spleen.	H	200 c.m. ser., S. R. C. / 50 c.m. ser. 5 / at 37 for 15 m. } + corps. 5=*Nil.*
6. Child, born in Duars. Very large spleen.	H	.....

XIII.—Condition of Blood in Native Coolie. Strong Antibody.

1. Coolie. Intense anæmia. Ascites. Dropsy of legs. Quartan parasites. { 150 c.m. ser., S.R.C. / 100 c.m. ser. 1 / at 37 for 15 m. } + corps. 1=No H Agg.

{ 150 c.m. ser., S.R C. / 10 c.m. ser. 1 / at 37 for 15 m. } + corps. 1=H after some hours.

{ 150 c.m. ser., S.R.C. / 2 c.m. ser. 1 / at 37 for 15 m. } + corps. 1=H after some hours.

To show that Class A blood may be associated with intense anæmia.

1. Coolie, intensely anæmic; œdema of face. Anchylostome ova in fæces :—

½ c.c. emuls. corps. + 150 c.m. ser., S. R. C. = No hæmolysis, no agglutination.
Ditto + 300 c.m. ser., S. R. C. = Ditto.
Washed corps. + Undiluted ser., S. R. C. = Ditto.

We have therefore a condition (A) seen in health, characterised by insusceptible corpuscles together with the presence of a natural hæmolysin, amboceptor *plus* complement, in the blood of the same person. This hæmolysin acts upon the susceptible red cells of other persons, therefore it is an isolysin.

On the other hand we have a condition (B) in which the red bloo corpuscles are susceptible to the isolysin referred to above. In this case there is not only an absence of isolysins directed against any of this (B) or the other class A blood, but there also exists in different degrees a powerful antibody, which appears to be of the nature of an anti-complement or anti-amboceptor directed against the complementophile group of the amboceptor. This antibody can neutralise the action of serum of class (A) upon susceptible corpuscles of class (B).

The exceptions to this general rule may be mostly embraced under the following additional generalisations :—

(1) A class (A) blood may possess little or no isolytic action. Examples of such bloods are No. 6 in series 2.

But very often if these sera are tested against very susceptible corpuscles they will give evidence of isolytic action.

(2) The serum of a not very susceptible class (B) blood may act upon the corpuscles of a very susceptible (B) blood. An example is seen in No. 3 series 2.

In only one instance No. 4 series 3 have we met a condition apparently diametrically opposed to the general rule.

Here not only were the corpuscles very susceptible, but the serum also acted upon class (B) corpuscles.

We appeared here to have a special isolysin in association with a blood of class B.

These facts appear to us to have some bearing upon the question of the existence of autolysins, for we have an isolysin which from its frequency in quite healthy conditions seems to be of physiological significance. For some reason many persons possess red cells which are susceptible to this isolysin and almost invariably the organism seems to have protected itself in these cases by the production of antibody, which will neutralise the isolysin.

But it is improbable that the organism would produce an antibody directed against a hæmolysin to which it had never been exposed so that the very existence of this anti body suggests the previous action on the blood of a hæmolysin with cytophile and complementophile groups similar to those of the isolysins present in the serum of class A bloods.

Our experiments show that the re-actions are not dependent upon race, age, sex, or upon the existence of mere anæmia. They also show that the condition

is not a transient one but remains the same in any individual case for long periods.

That class A is the normal condition is suggested by the fact that six European visitors all had class A blood, and in one case of class B blood we have seen a slowly diminished susceptibility follow quinine treatment and recovery from a condition of marked malarial anæmia.

But the number of persons with class B blood seems to preclude any direct relation to malaria.

Nevertheless as will be seen from the following observations there is a remarkable preponderance of class B over class A bloods among European residents in the Duars

Table XIV.—Showing the relative frequency of class A and class B blood in various communities.

	Class A.	Class B.
Non-resident Europeans who there is every reason to believe have not recently suffered from malaria or been exposed to infection.	6	...
Europeans, resident 3 months to 5 years in the Duars suspected or known to have suffered from malaria recently or to have been exposed to infection.	...	13
European, over 5 years' resident in the Duars, many of whom are known to be exposed to infection.	5	11
Europeans, resident in the Duars little exposed to infection, apparently in good health, taking quinine or otherwise escaping malaria.	4	2
Babus, new-comers and old residents in the Duars in various conditions as regards health.	6	18
Other native residents of different races including Chinamen, Paharias, Sikh, and Beharis.	4	3
Chota Nagpur coolies in the Duars resident 1—3 years.	2	3
Natives in Jalpaiguri.	2	9
English Soldiers, Calcutta	23	26
English Sailors, Calcutta.	2	4
Natives, Calcutta Jail	7	28
Europeans, resident in the Duars	9	26
Europeans, non-resident in the Duars	31	30
Natives, resident in the Duars.	14	33
Natives, non-resident in the Duars	17	28

In our experiments we have laid stress on the occurrence of hæmolysis, but the observations apply equally to agglutination so that in the vast majority of cases if we know with what class of blood we are dealing we can deduce whether or not, in any given case, agglutination will occur. It is obvious that work such as that of Capograssi's who investigated the agglutinating action of the sera of malarial subjects must be enormously modified by knowledge of the facts we have set forth.

Whether these facts have any relation to the conditions which give rise to Black-water Fever we are unable to say. The change from a state of class A to one of class B blood seems to us to be fraught with possible danger, and residence under Black-water Fever conditions seems associated with the occurrence of this change. In Europeans also the amount of anti-hæmolysin present under the conditions we have investigated is much less than that usually found in natives, especially those with the most markedly susceptible corpuscles.

At present the most important application of these results is that, in attempting to demonstrate hæmolysins in disease, it is necessary to give due consideration to the conditions we have indicated.

Chapter IV.

The Nature of the Black-water Fever Process.

All our researches point to Black-water Fever being the effect of poisoning by a hæmolysin.

Nor does it seem to us that this hæmolysin poisoning should be conceived of as some vague process spoken of without recognition of the method of action of these poisons.

We have already seen that in Black-water Fever the blood destruction is mainly of that nature to which we have given the name lysæmia.

It is not simply the action of a hæmolysin but a hæmolysin acting under special conditions. The fact that in Black-water Fever the hæmolysin has not been demonstrated, is for reasons we have shown not remarkable. For in the case of dogs actually injected with hæmolytic serum it is quite impossible with the knowledge we possess to demonstrate, apart from its effects, the presence of the substance (hæmolysin) that we have injected; though in vitro its presence is easily demonstrable.

As regards the hæmoglobinuria in Black-water Fever we have seen that to look upon the presence of hæmoglobin in the urine as an indication of the amount of blood destruction is quite misleading; that hæmoglobinæmia does not necessarily result from even the greatest amount of blood destruction; that in malaria although an enormous amount of blood may be destroyed this is mainly due to the corpuscles being retained and broken down in the organs and not to their solution in the plasma; and that for hæmoglobinuria to ensue only a trace of hæmoglobinæmia is necessary. We have seen also that this hæmoglobinæmia is a superimposed condition of blood destruction, characterised by solution of the red cells or lysæmia; that this is not due to osmotic variations in the plasma, to changes in the resistance of the red cells to salt solutions or to invasion of these cells by any parasite. Coming to broader issues we have seen that Black-water Fever results from a condition induced by repeated attacks or infections by malaria; that it is precipitated by an acute attack of malaria, especially when under certain conditions this is associated with the administration of quinine.

We have seen that quinine in itself is innocuous and cannot have such an effect on a subject not previously prepared; that the consistent use of the remedy by a community may even diminish the incidence of Black-water Fever by reducing the liability to malarial infection.

The production of the hæmolysin we have seen is in some way the result of malaria, and it is generally considered by those who take the malarial view that it is in the form of a toxin derived from the malarial parasite.

Such a toxin has never been demonstrated and the fever and other symptoms of malaria are explicable without assuming its presence. Also if such a toxin were the cause we should not expect length of residence to play so important a part in the ætiology of the disease, for there seems no reason why the action of quinine in a severe attack of malaria, in a new-comer, should not bring about the liberation of a hæmolysin from the malarial parasite, with resulting Black-water Fever; the length of residence suggests changes produced in the body itself.

It is desirable to consider what are the possible changes that long-continued malaria might bring about. In the injection of goat's blood into goats for short periods Ehrlich was once able to demonstrate an autolysin.

It is by no means improbable that malaria which gives rise to periodical destruction of red cells might have the same effect in man. No other pathological condition known to us is so likely as malaria to give rise to an autolysin. It, more than any other disease or experimental condition, demands of the organism the repeated destruction and the absorption of its own red cells.

We have seen that there is a close resemblance between Black-water Fever and paroxysmal hæmoglobinuria; both are conditions of unstable equilibrium, the one apparently natural to the subject, the other induced; in both the exciting causes are such as under normal conditions produce no effect.

In paroxysmal hæmoglobinuria mere local cold is sufficient to cause hæmoglobinuria. This is an indirect action, for cold acting upon blood has no such effect; and the hæmolysin responsible for the blood destruction must therefore be thrown out by the body cells in response to stimulation.

Such considerations suggest reasons why the malignant tertian parasite should be chiefly concerned in Black-water Fever. For it is in infections by the malignant tertian parasite that we find the organs most actively engaged in holding up the infected red cells, the very perniciousness of infections by this parasite being suggestively bound up with such an action.

If we conceive of the hæmolysins being derived not from the parasite but thrown out as a result of the constant phagocytosis of red cells, we shall also understand better why it is that the apparently robust should suffer. Able to make good the loss of his red cells the actual infection by the parasites affects the well-fed adult European or better class native but moderately and the very power of reaction which we know to be associated with healthy rather than diseased conditions may be his undoing.

If hæmolysins are formed against the blood there seems no agent so likely to effect this as the endothelium which everywhere is the tissue in contact with the blood and is largely concerned in regenerative and phagocytic functions.

This view receives support on a consideration of certain conditions associated with paroxysmal hæmoglobinuria, Raynaud's disease in particular, where local vascular spasm is connected with the occurrence of the disease.

In this connection malaria again comes prominently to the fore as a factor likely to have a marked effect upon the vascular endothelium. For months and years this tissue has to experience the constant association with altered and broken down red cells and parasites, with consequent stimulation to active phagocytosis of these bodies.

Our work shows, however, that whatever the origin of the hæmolysin (hæmolytic amboceptor) may be we have still to ascertain why the lysæmic condition results. If we consider the possibility of its being due to the sudden liberation of complement in the living body many otherwise inexplicable facts become readily understandable, but such an hypothesis at present rests upon no actual data.

In few diseases do we know the ultimate working of the morbid process, so that because we do not yet know the whole mechanism of Black-water Fever it does not follow that we are totally ignorant of the nature and origin of the disease.

Our researches we think have helped to make this nature of the disease clearer than it has hitherto been. We have as a result of our observations come to a truer conception of the conditions under which the hæmoglobinuria of Black-water Fever occurs, than has previously been possible; and this conception of the disease is a strong incentive to further experimental work.

Part V.

Prophylaxis and Treatment of Black-water Fever.

We have still to discuss what steps are possible in regard to the prophylaxis of Black-water Fever and in regard to the treatment of the condition when it has once arisen.

Prophylaxis.—If our conclusions as to the origin of Black-water Fever be correct the prophylaxis of the condition is simply the prevention as far as possible of malarial infection, and the prompt and efficient treatment of this disease, with the view to prevent the occurrence of relapses.

It would be foreign to our purpose to enter fully into the large question of anti-malarial sanitation in this memoir but it will not be without interest to ascertain as far as possible whether in actual practice there are any examples of a reduction in the incidence of Black-water Fever due to the adoption of proyhylactic measures directed against malaria, especially to the active pushing of quinine, the very drug which is generally looked upon as largely responsible for the occurrence of Black-water Fever.

According to Deaderick (92) such a reduction is reported by Mercier in German East Africa, by Ziemann in the Kameroons, by A. Plehn in Togo and by Kohlbrugger in the Malay Archipelago.

Quinine prophylaxis, it is obvious, to be of any use must be effectively pushed. In the Duars already a large proportion of the European population has taken to the systematic use of quinine. The result has so far been not only a much reduced amount of malaria amongst those taking the precaution but a very small number of cases of Black-water Fever in this community.

The only three cases which occurred among Europeans during the past twelve months were among those who professed not to believe in quinine and only took it when they felt unwell. Whether the explanation we have given be the correct one will be shown in time.

Treatment.—It is not our intention to touch upon the ordinary palliative treatment of Black-water Fever. Our object is to show that there are excellent reasons for believing that good results may be expected from serum therapy.

We have shown that a toxin derived from the malarial parasite is an unlikely cause of Black-water Fever and that the active agent is probably one derived from auto-immunisation against the organism's own red cells.

The attempt to produce an anti-amboceptor against the cytophile group of a hæmolysin which we are unable to isolate seems to us at present useless. It is otherwise, however, as regards the production of an anti-complement or an anti-amboceptor directed against the complementophile group. Ehrlich in his studies upon complement has come to the conclusion that these exist in any serum in

great variety. Observations upon the absorption of complement show, however that in spite of this multiplicity, the whole of the complement in a serum may be abstracted by a single type of amboceptor, provided that its cytophile group is able to combine with a suitable receptor. The received explanation of these apparently contradictory results has led to a further modification in the coception of the amboceptor and complement. In a recent paper Ehrlich and Sachs (93) conceive of the amboceptor as having a single specific cytophile group but a large number of complementophile groups which vary considerably in detail but in their entirety represent a uniform complex. *This complex is reproduced in all amboceptors of the same serum.*

Anti-amboceptors directed against the complementophile groups and obtained through immunisation with any particular amboceptor will act against all amboceptors of the same animal species, no matter whether these amboceptors are normally present in the serum or have been produced by immunisation.

Immunisation of small animals with human serum should offer no insurmountable difficulty, but until a considerable number of experiments have been carried out it is not possible to say whether by such means we might be enabled to produce an anti-amboceptor or anti-complement suitable for use in the treatment of Black-water Fever.

That treatment on these lines is not chimerical is shown by the work of Widal and Rostaine (62) on paroxysmal hæmoglobinuria, who made use of a serum obtained by the immunisation of a rabbit against human serum. The administration of this serum to the subject of the paroxysmal hæmoglobinuria served to counteract the effects of exposure to cold which previous to its use was invariably followed by hæmoglobinuria.

In Black-water Fever we have a condition often lasting several days during which the blood destruction steadily continues. To be able to check this destruction early in the disease would be no small achievement and enormously increase the chance of a favourable termination.

The feasibility of these suggestions, however, can only be shown by further experiment.

PART VI.

APPENDIX.

RECORD OF CASES INVESTIGATED.

Case I.—Chengmari Garden. August 1907.
Bengali Babu.

Seen on 3rd day. Hæmoglobinuria over. No malarial parasites. Scanty pigmented leucocytes. Decolourised corpuscles in cells of mononuclear and macrophage type. Leucocytes.

	Per cent.
Polymorphonuclears	57
Large mononuclears	24·9
Small mononuclears	16·6
Eosinophiles	1·5

Macrophage type conspicuous.

Case II.—Chengmari Garden. August 1907.
Uriah mali. Age 35.

Native country.—Cuttack.

Length of residence in Duars.—3 years.

History.—Had suffered from fever and enlarged spleen. Was treated for about a month, but has had no treatment for last two months.

On 28th July got high temperature with diarrhœa, bloody urine and vomiting of green bile.

Seen by Dr. Babu on 30th July. He then had suppression of urine. Died 31st July.

Autopsy.—Liver very congested, enlarged, granular and friable.

Spleen much enlarged, soft, tumid, and greatly congested.

Kidneys greatly enlarged and *dark purple in colour.*

Much malarial pigment in macrophages and mononuclear cells of liver and spleen.

Many macrophages and large mononuclear cells in the liver loaded with greenish pigment. Liver cells themselves contain similar greenish pigment. Sections shew many engulfed red cells lying in large macrophages.

Case III.—Meen Glas Garden. July 1907.
Babu's servant.

History.—Came on 26th July to Meen Glas looking for work. Was obviously very ill and was sent by the manager to the Dr. Babu. Was given 10 grains of quinine, mid-day, 27th July. When seen that night he had high fever, vomiting and some jaundice. Urine was then seen to be black. Died 28th July.

Autopsy.—Made by Dr. Babu who supplied us with portions of the liver, spleen and kidneys.

In films macrophages and mononuclear cells are seen loaded with malignant tertian pigment scattered and in blocks. In sections immense numbers of engulfed red cells are seen in the macrophages of the spleen and liver but especially in the former, red cells are also seen in small cells lying in the venous sinuses of the splenic pulp. Almost every macrophage in the spleen contain also one or more nuclear masses which seem to be engulfed and altered portions of leucocytes; these masses stain very intensely but often shew a somewhat clear central or eccentrally placed space. Yellow or greenish-yellow pigment is seen especially in large macrophages in the hepatic capillaries and in the liver cells.

The hepatic veins contain much granular matter.

Case IV.—Chalouni Garden. August 1907.

Dhoby. Age 21.

Native country.—Muzuffarpur.

Length of residence in Duars.—Nine months.

History.—Was noted to be suffering much from fever by Dr. Babu in April. His spleen was found enlarged in June, but may have been so earlier.

On *11th August 1907.*—Complained of fever. Was given quinine, but it is uncertain if he took this.

On *15th August 1907.*—Again complained of an attack of fever, and is stated to have swallowed two tabloids of 5 grains each quinine sulphate at 9-30 A.M., but to have vomited these undissolved at 10 A.M. He was noted on this morning to be very anæmic, reduced, and jaundiced. At 6 P.M. the urine brought to the Dr. Babu was dark-red. He states that the first red urine was passed at 2 P.M.

7 p.m.—Temperature 104·8. Pulse 174. Respirations 44. Spleen and liver enlarged.

12 Mid-night.—Temperature 106. Pulse 182. Respirations 56. Thirst.—Vomiting. Later unconscious.

5 a.m.—Temperature 100. Pulse 96. Respirations 26. Blood showed many pigmented leucocytes. No parasites on first examination, but one small malignant tertian *accole* form found subsequently.

17th August 1907.—Red cells 942,000. Hæmoglobin 15 per cent. A few pigmented leucocytes still present.

Blood—

16th August 1907.—Leucocytes counted 700.

	Per cent.
Polymorphonuclears	59
Large mononuclears	20·5
Small mononuclears	20
Eosinophiles	·5

Macrophage type abundant.

Case V.—Meen Glas Garden. August 1907.

Bengali Babu.

Length of residence in Duars.—Twenty years.

History.—Has been 7 years at Meen Glas. Had lately been having fever and was getting 10 grains quinine a week for this. Ten days prior to attack had fever.

19th August 1907.—Was given 10 grains quinine; the same day he had a slight rigor, vomiting, and passed dark urine which contained hæmoglobin. He refused to believe he was suffering from Black-water Fever because his urine was not exactly "the colour of ink."

Seen by us within a few hours of the onset of the attack. Examination of his blood showed a few malignant tertian rings and many leucocytes showing included red cell shadows. Twelve hours later the number of parasites was much reduced.

On the day following the attack no parasites were found. Hæmoglobin was 44 per cent. Red cells 2,460,000.

Ten days later he had a malarial attack with malignant tertian parasites which was successfully treated with quinine.

Conditions under which the case occurred. The patient's house was close to extensive coolie lines. For some months his children had been living with him. Both were infected, one with malignant tertian and one with simple tertian parasites.

Blood—

19th August 1907.—Leucocytes counted 500.

	Per cent.
Polymorphonuclears	49·7
Large mononuclears	26·3
Small mononuclears	23·8
Eosinophiles	·2

4 cells containing shadow corpuscles. 48 pigmented cells of which about one-third are small cells resembling small mononuclear leucocytes.

One small malignant ring parasite.

17th August 1907.—Leucocytes counted 550.

	Per cent.
Polymorphonuclears	54·3
Large mononuclears	27·4
Small mononuclears	18·3
Eosinophiles	0

4 cells containing shadow corpuscles.

87 pigmented cells.

No parasites.

18th August 1907.—Leucocytes counted 600.

	Per cent.
Polymorphonuclears	55·6
Large mononuclears	21·6
Small mononuclears	22·8
Eosinophiles	0

A few cells with included shadow corpuscles and a few pigmented leucocytes only.

19th August 1907.—Leucocytes counted 600.

	Per cent.
Polymorphonuclears	53·6
Large mononuclears	16·6
Small mononuclears	2·4
Eosinophiles	5·6

A few pigmented cells. Many small mononuclears show an accessory nuclear (?) mass.

Case VI.—Meen Glas Garden. August 1907.

Mohamedan bearer.

Native country.—North-West Provinces.

Length of residence in Duars.—Two years.

History.—States that he has had no fever this year though he previously had a good deal. The manager states that he has had fever. Was taking 10 grains of quinine a week.

Friday night had a little fever.

Saturday took his weekly dose of quinine. In the evening had a slight attack of fever but did his work.

Sunday morning the Dr. Babu gave him 10 grains quinine. His master seeing that he had fever gave him another 10 grains quinine. About mid-day he had a slight chill and passed dark brown urine containing hæmoglobin. Hæmoglobinuria was over in less than twenty-four hours. Temperature never rose above 100.

Seen by us on the following day. Spleen enlarged. No malarial parasites. Four pigmented leucocytes found in a count of 500 cells.

Was given quinine subsequently without return of condition.

Case VII.—Nagasuri bazaar. September 1907.

Bengali shopkeeper.

Length of residence in Duars.—Five years.

History.—Had been getting much fever and went to Calcutta for a change. On returning was taken with fever in the train, and took 10 grains of quinine. A few hours after passed dark urine.

Seen by us on the third day when hæmoglobinuria had ceased. Blood showed pigmented leucocytes. A few red corpuscle shadows in leucocytes A count of 500 leucocytes gave as follows:—

	Per cent.
Polymorphonuclears	53·8
Large mononuclears	28·5
Small mononuclears	17·2
Eosinophiles	·2

Case VII.—Bagracote Garden. September 1907.
Bengali Babu.

History.—Was at Chalouni for five years. Went to his country for several months, and on returning to the Duars went to Bagracote, where he had been living for three months before his attack. Whilst at Bagracote had a good deal of fever. Had an attack of fever a fortnight before the Black-water Fever for which he had taken quinine after the fever was over.

Since Friday had had fever. On Sunday for the first time took 15 grains quinine. Quinine was taken 7 A.M. At 11 A.M. had a severe rigor, vomiting, and passed very black urine.

Seen by us on first day. His blood taken within four hours of the onset showed malignant tertian rings. Later on on the same day no parasites could be found. During the attack the *spleen underwent considerable enlargement* and was very painful. Epigastric pain very marked and vomiting very severe.

Hæmoglobin when first seen 70 per cent. Following day it was 30 per cent. and subsequently at the cessation of the Black-water Fever it was still lower.

Hæmoglobinuria ceased on the third day and the urine became clear. Suppression of urine then supervened. Death followed. No autopsy.

Blood—

1st day Leucocytes counted 500.

	Per cent.
Polymorphonuclears	45·7
Large mononuclears	23·1
Small mononuclears	32·2
Eosinophiles	0

Malignant tertian parasites and a few pigmented leucocytes. A few red cell shadows in mononuclear cells.

2nd day Leucocytes counted 500.

	Per cent.
Polymorphonuclears	60·9
Large mononuclears	18·1
Small mononuclears	21·9
Eosinophiles	·1

No parasites. Pigmented leucocytes and cells containing red blood corpuscles more numerous than on first day.

Condition under which attack occurred.—His wife and three children had been with him for a fortnight. Two of the children were infected. The house abutted on coolie lines. Twenty-five children whose bloods were examined were all infected, eleven showing simple tertian, thirteen quartan, two malignant infections. Fifty yards away a Paharia child was found whose blood was swarming with crescents.

Case VIII.—(*a*)

Dhoby.—Living within one hundred yards of where the last case occurred was noticed to be suffering from anæmia with a temperature of a 100°F. Examination of the blood showed nucleated red cells. No parasites were present in spite of the fact that he had not been getting quinine. His urine contained albumen.

History.—He had come to the garden somewhat over a year ago. Up to ten days ago he had been doing his work though on enquiry it was found that he had suffered from frequent attacks of fever. A few days before he was seen by us he was seized with shivering bilious vomiting and passed dark urine. He died two days later.

Case VIII.—(*b*)

On the railway line in the garden a Paharia boy intensely anæmic and jaundiced was found dead by the police.

Case IX.—Ranachera Garden. September 1907.

Chinaman.

History.—Has had two previous attacks of Black-water Fever. Had fever for thres days. On the second day took quinine. The next day no quinine. The day following at 10 A.M. took 10 grains quinine. At twelve noon had a slight rigor and at 2 P.M. passed black urine.

Seen by us at 4 P.M. *Spleen not palpable at this time. Five hours later the spleen was two finger breadths below the costal margin. The next morning it was a hands-breadth below.* There was marked præcordial pain and breathlessness.

A single small malignant ring found after a long search and few pigmented parasites. A count of 500 showed:—

	Per cent.
Polymorphonuclears	66·3
Large mononuclears	27·8
Small mononuclears	4·7
Eosinophiles	1·2

Second day.—Hgbn. 55 per cent. Red cells 2,560,000. White cells 8,690.

Hæmoglobinuria continued for three days followed by fever for about a week. After an apyretic period more fever occurred, but no parasites could be found at this time. Quinine also had no effect on this fever. After eight or nine days during which he was still getting fever he went to Calcutta.

Case X.—Jalpaiguri. September 1907.

Police Constable.

Length of residence in Duars.—On survey duty in Duars for six months.

History.—Himself, wife, and daughter all suffered much from fever. States that as a result of taking quinine he had freedom from fever for a time. A month before his attack he began to suffer again. Ten days before his attack he had fever, and also for the three days preceding the Black-water Fever. On account of a police case he had to go up to Jalpaiguri. About 7 A.M. on the day of his case he took quinine. Going to the police station he was taken ill, about 11 A.M., with Black-water Fever.

Seen by us on second day. No parasites but two pigmented cells seen in counting 500 leucocytes.

A count of leucocytes gave :—

	Per cent.
Polymorphonuclears	48·4
Large mononuclears	26·6
Small mononuclears	24
Eosinophiles	1

Case XI.—Monabari Garden. October 1907.

Bengali Babu. Age 50.

Native country.—Dacca.

Length of residence in Duars.—18 years.

History.—Before coming to Monabari he was seven years at Bagracote where he states he did not get much fever. During the three months he spent at Monabari he had constant fever, not less than two attacks every month.

Black-water Fever occurred on the evening of Saturday six hours after taking 10 grains of quinine. Urine at first was very dark but it cleared up in less than twenty-four hours.

Seen by us on the second day sixteen hours after the onset of the attack. No malarial parasites but 15 pigmented cells seen in a count of 500 leucocytes. Cells containing included red cell shadows were also present.

Leucocytes were as follows :—

	Per cent.
Polymorphonuclears	61
Large mononuclears	33
Small mononuclears	6
Eosinophiles	0

Had a second attack six months later. *Vide* Case XXVII.

Case XII.—Pathajora Garden. October 1907.

Malpaharia Sirdar.

Native country.—Santal Parganas.

Length of residence in the Duars.—15 years.

History.—Had been working lately at water works at the foot of the Pathajora gorge. He got severely chilled and got bad fever for which he was given

quinine. There was no history of a rigor, but severe vomiting and for twenty-four hours he passed reddish black urine.

Seen by us on the third day when the hæmoglobinuria was over. He was anæmic and slightly jaundiced. The temperature was still high. The spleen was enlarged. No parasites and no pigmented leucocytes. Engulfed shadow corpuscle present.

A count of leucocytes gave:—

	Per cent.
Polymorphonuclears	49
Large mononuclears	24·6
Small mononuclears	21
Eosinophiles	5·4

Case XIII.—Nagrakata Garden. October 1907.

European child. Age 3 years.

History.—Had two attacks one on the 14th October and one on the 22nd October, in both of which there was very transient hæmoglobinuria and the child very slightly ill. It being noticed that the urine was red samples were on each occasion sent to Dr. Stone who noticed the nature of the condition.

Blood examined on the first day showed quartan parasites and pigmented leucocytes.

Case XIV.—Jalpaiguri. November 1907.

Police Constable. Second attack following that recorded as Case X.

Seen by us on the third day. No malarial parasites. Four pigmented leucocytes seen in a count of 500 cells. Engulfed red cells including one apparently unaltered present in mononuclear cells.

Leucocytes count as follows:—

	Per cent.
Polymorphonuclears	59·4
Large mononuclears	26·4
Small mononuclears	8·6
Eosinophiles	5·6

Case XV.—Moortee Garden. February 1908.

Bengali Babu.

Length of residence in Duars.—7 years.

History.—States that his wife and himself had been constantly getting fever.

Seen by us on 4th day when hæmoglobinuria was over. Urobilin present in urine. No malarial parasites and no pigmented leucocytes. A few engulfed red cell shadows seen in mononuclear cells.

Leucocytes count gave:—

	Per cent.
Polymorphonuclears	54·2
Large mononuclears	24·8
Small mononuclears	20·8
Eosinophiles	·2

His wife seen at the time had also urobilin in her urine.

Case XVI.—Chota Nagpur. December 1907.

European. Age 27.

In "Timber."

History.—Formerly a planter in the Duars. Has lately been living at Kalunga above Purulia, Chota Nagpur. Was quite well up to ten months ago when he began to get fever characterised by vomiting. At first these attacks lasted three or four days. They then became more frequent but the attacks were not so bad. Had fever a few weeks before his present attack. On the night of his attack he was travelling to Calcutta, and, feeling seedy, took quinine. This was about 9 P.M. In the night he was attacked with Blackwater Fever, and was admitted into hospital next morning. Temperature 104. He exhibited all the characteristic signs of the disease. Urine hæmoglobinous. Spleen 3½ inches below the costal margin.

Seen by us after the hæmoglobinuria was over, but two films taken during the hæmoglobinuria were kindly given to us by Major Rogers, I.M.S.

Blood.—No malarial parasites or pigmented leucocytes. Occasional included red cell shadows seen in mononuclear cells. Four nucleated red cells seen whilst counting 800 leucocytes.

A count of 2,000 leucocytes gave:—

	Per cent.
Polymorphonuclears	56·3
Large mononuclears	17·7
Small mononuclears	25·3
Eosinophiles	·4

Associated conditions.—One other European lived at Kalunga. He also was stated by the patient to suffer from constant fever.

Case XVII.—Baradigi Garden. December 1907.

Bengali Babu. Age 28.

Native country.—Ferakpur.

Length of residence in Duars.—8 years with intervals on leave at home.

History.—Had "jaundice" in the Duars four years ago. This came on suddenly after fever and left him very weak. Last return from home December 1906. Was ten months in Central Duars where he only remembers having fever once. He has been six wees at Baradigi where he states he has had no fever. On 13th December 1907 was feverish, and was given 15 grains of quinine on this and the following day. This last dose he took about 3 P.M. On 16th December 1907 he felt fairly well, until shortly before 3 P.M. when he passed some reddish black urine. With this he had "ague," headache, and a temperature of 104. He passed dark urine four times during the night.

On morning of 17th December 1907 his temperature was 99 and the urine was clear but with a sediment. Early in the morning was given 5 grains quinine. He

was seen about 11 A.M. by Dr. McCutcheon who took blood films and noted the temperature to be 103. About 12·30 P.M. the patient is stated to have passed some more slightly red urine.

On morning of 18th December 1907 the temperature was normal.

Seen by us on the 19th. Mucus membranes anæmic, yellowness of conjunctivæ distinct. Spleen three fingers-breadth below costal margin.

Blood.—Isotonic point $\frac{N}{13}$. No malarial parasites, but pigmented leucocytes present. On the 19th large endothelial plaques and mononuclear cells, many of them containing shadow corpuscles, were very conspicuous.

Leucocyte count gave:—

	17th December 17. Per cent.	*19th December 1907.* Per cent.
Polymorphonuclears	48·9	62·3
Large mononuclears	30·8	22·3
Small mononuclears	20·1	12·7
Eosinophiles	·2	2·4

Case XVIII.—Baradigi Garden. December 1907.
Bengali Babu. Age 30.

Native country.—Nadya.

Length of residence in Duars.—9 years.

History.—No history of previous attacks. Has had enlarged spleen for ten years or more. Was recently home for eight months. Has been back in the Duars for 15 months, six at Sissibari, one at Bentguri, some months near Darjeeling, and two months at Baradigi. Since return from home has been getting fever once a month or so. At Sissibari had five attacks.

13th December 1907.—Felt a little fever and ague for which he took 10 grains of quinine.

14th December 1907.—Felt all right.

15th December 1907.—Was all right and working in the tea-house, but at 3 P.M. passed reddish black urine and had ague. His temperature shortly after was 105. He passed dark urine three times up to 10 P.M.

16th December 1907.—Urine clear. Temperature in the morning 97, at evening 100.

17th December 1907.—Seen by Dr. McCutcheon, who took a blood film. His temperature on this and the next day was normal in the morning but rose to 100 in the evening.

Seen by us 19th December 1907.—Anæmia not marked. No distinct yellowness. Spleen five fingers below the costal margin. Blood taken on the 17th showed no parasites or pigmented cells, nor were engulfed shadow corpuscles seen. Large mononuclears numbered in a c1 of 1,200 leucocytes 18 per cent. Some myelocytes were present.

Case XIX.—Bentguri Garden. January 1908.
Mahomedan Bearer, age 32.

Native country.—Mongyr District.

Length of residence in Duars.—12 years.

History.—Went home on leave on account of fever and spleen. Suffered severely whilst at home from fever. Came back to Bentguri five months ago, and has been living at the Assistant's bungalow, until six weeks ago when he moved with his master to the Manager's bungalow.

He is believed by his master to have had previous attacks, but he himself denies this.

28th December 1907.—Had slight fever and took 10 grains of quinine. States he has had no quinine since except two doses of spleen mixture which he took four days before the onset of the present attack.

7th January 1908.—In the evening about 6 P.M. had a little fever and was given 10 grains phenacetin by his master.

8th January 1908.—In the morning felt all right and worked till 10-30 A.M. At this time he had a severe rigor lasting about two hours with an intermission during which he sweated profusely. Urine passed at 1-30 P.M. and in the afternoon hæmoglobinous. His temperature at 2 and at 4 P.M. was 102.

Seen by us about 8 P.M. Spleen two finger-breadths below costal margin. Temperature 101. Splenic puncture gave a small quantity of blood very rich in cells.

Blood.—Serum separated from clot, deep yellow colour, with a reddish tinge. No solution of the clot in serum, or deepening of red colour of serum took place even after many hours. On morning of 9th January 1908 serum is lighter in colour. Hæmoglobinæmia doubtful.

Isotonic point on morning 9th :—$\frac{N}{15}$

	Per cent.
Hæmosozic value at 9 A.M., 9th January 1908 . .	1·06

Films of peripheral blood taken 8 P.M. on 8th January 1908 show no parasites and no pigmented leucocytes. No parasites or pigmented leucocytes were found in the splenic films.

A count in each case of 600 gave as follows :—

	8 P.M. 8th January 1908. Per cent.	9 A.M. 9th January 1908. Per cent.	4-30 P.M. 9th January 1908. Per cent.
Polymorphonuclears . .	83	67·5	54
Large mononuclears . .	9·6	8	13
Macrophages . . .	1·3	...	...
Small mononuclears . .	6	18	30
Eosinophiles . . .	0	6·5	3

Measurement of red cells showed as follows :—

	8 P.M. 8th January 1908. Per cent.	4-30 P.M. 9th January 1908. Per cent.
Large cells measuring 140	49	17·5
Medium cells measuring 120 . . .	46	70
Small cells measuring 100	5	7·5

Examination of splenic films showed two somewhat doubtful malignant rings but no pigment. The following type of cells are distinguishable :—

1. Large macrophage type. Protoplasm light coloured containing small vacuoles, diffuse reddish stippling and a few larger reddish granules. Many of these cells contain decolourised red cells and greenish masses. Throughout the film are many globular masses formed of detached protoplasm of this and the next type of cell.

2. Large cells with rounder nuclei and with protoplasm which takes a deeper stain and is firmer in consistence.

3. Small mononuclear cells with voluminous clear hyaline protoplasm, similar to small mononuclear leucocytes.

4. Small mononuclear cells with scanty deeply staining protoplasm. Lymphocyte type.

5. Still smaller cells with very deeply staining protoplasm containing a few red granules.

6. Polymorphonuclear leucocytes and transitional cells.

Some stromata of red cells are seen resembling those sometimes present when blood is treated with hypotonic salt solutions.

Hæmolysis experiments.

Black-water Fever serum .	normal corpuscles . .	normal serum *Nil.*
„ „ „ .	washed normal corpuscles . . .	*Nil.*
„ „ „ .	washed corpuscles of a malarial subject.	. Marked agglutination.
Black-water Fever corpuscles	washed serum of malarial subject . .	*Nil.*

Urine—

First sample passed 1-30 P.M., 8th January 1908.—When first seen a somewhat impure rosy red. Showed marked oxyhæmoglobin bands with a faint band in the red and a broad band in the blue end of the spectrum. Next morning the band in the red (indicating met-hæmoglobin) was much more marked.

Second sample dark brown hæmoglobin bands present with distinct band in the red.

Hæmosozic value of urine :—

	Per cent. salt.
Urine passed, 1-30 P.M., 8th January 1908 . .	·251
Urine passed, morning, 9th January 1908 . .	·834

Associated conditions.—The patient was one of four servants who had been living with their master at the Assistants' bungalow, but had recently come to live in the servant lines at the Manager's bungalow, the master having changed his quarters temporarily on account of constant fever.

The Assistants' bungalow was situated in the bend of a small rivulet swarming with anopheles and within 50 yards of large cooly lines.

The condition of the other people living here was :—

Mr. J.—Had fever recently, is taking quinine, blood anæmic.

Bearer.—Intensely anæmic. Enlarged spleen. Simple tertian infection.

Syce.—Intensely anæmic. Enlarged spleen. Simple tertian infection.

Waterman.—Fever at the time. Scanty malignant tertian rings. Enlarged spleen.

It was evident that all these people at the Assistants' bungalow had been subjected latterly to malarious conditions of great intensity.

Case XX.—Hyatpatha Garden. January 1908.

Bengali Babu. Age 20.

Length of residence in Duars.—5 years.

History.—No history of previous attacks. Has been ten months at Hyapatha. Three months ago had "liver" and was yellow in the eyes. He has had enlarged spleen for one year.

13th January 1908.—At 11 A.M. had fever with ague and nausea. Did not attend work after this on account of sickness.

14th January 1908.—Took three tabloids of quinine, 5 grains each.

15th January 1908.—Took four tabloids of 5 grains each.

16th January 1908.—Took two tabloids at 8 A.M. and a dose of spleen mixture at 9 A.M. At 10 A.M. had ague. At 2-30 P.M. passed dark red urine and continued to pass urine of a dark ruby red all day.

Seen by us at 7 P.M. Complains of epigastric pain. Spleen below costal margin. Pulse full and of high tension. Coagulation of blood markedly increased. Temperature 104·8.

Splenic puncture was performed within a few minutes of our arrival.

Blood—

Coagulation markedly increased.

Hæmoglobinæmia shown by receiving blood into one per cent. salt and centrifugalisation.

Serum separated from clot of an intense orange colour, bands of oxyhæmoglobin being visible on spectroscopic examination.

Isotonic point—

$\frac{N}{10}$	$\frac{N}{12}$	$\frac{N}{13}$	$\frac{N}{14}$	$\frac{N}{15}$
tint	tint	tint	increased tint	marked H.

Hæmosozic value.—1·12 per cent. salt.

17th January 1908.—Passed hæmoglobinous urine all day.

Isotonic point.—9 A.M.

$\frac{N}{10}$	$\frac{N}{12}$	$\frac{N}{13}$	$\frac{N}{14}$	$\frac{N}{15}$
tint	tint	increased tint	increased tint	marked H.

Hæmosozic value.—1·258 per cent. salt.

Coagulation not so noticeably rapid as yesterday.

Serum separated from clot markedly yellow with orange tint. Hæmoglobinæmia shown by receiving blood into $\frac{N}{10}$ citrate and centrifugalising.

Temperature varies between 101·6 and 104, falling after 5 P.M. to 98·8.

18th January 1908.—Hæmoglobinous urine passed up to 12-50 P.M. After this the urine contained albumen and urobilin but no hæmoglobin.

Isotonic point.—9 A.M.

$\frac{N}{12}$	$\frac{N}{13}$	$\frac{N}{14}$	$\frac{N}{15}$	$\frac{N}{16}$
tint	tint	h	hh	inc H.

15 per cent. 40 per cent.

Hæmosozic value.—1·02 per cent. salt.

Coagulation delayed. Buffy coat present.

Hæmoglobinæmia not demonstrable. Yellow coloration much less intense.

Spleen about the same size. No noticeable enlargement of liver.

19th January 1908.—Urine contains much pink urates. No hæmoglobin but much urobilin albumen present. Blood shows 17 per cent. large mononuclears and many endothelial plaques.

Isotonic point.—6 P.M.

$\frac{N}{12}$	$\frac{N}{13}$	$\frac{N}{14}$	$\frac{N}{15}$	$\frac{N}{16}$
Nil	*Nil*	h	hh	inc H.

22nd January 1908.—After several days convalescence temperature rose this afternoon to 100 and the urine became brown, but no urobilin could be detected. Spleen only just palpable. Polychromasia and presence of large cells extremely conspicuous. No mononuclear increase. Some leucocytosis.

Went home for 21 days after his attack and was seen again on 20th May 1908. States that he had no fever until the previous day. Fever commenced at 10 A.M. continued all night until 3 P.M. yesterday at which time he took 15 grains quinine.

20th May 1908.—No noticeable anæmia. No enlargement of the spleen to be made out. No parasites.

Isotonic point—

·476 per cent.	·454 per cent.	·434 per cent.	·4 per cent.
Nil	*Nil*	trace	hh

Emulsion of washed corpuscles hæmolysed by Class A serum. Antibody 20 c.m. not quite protective against 150 c.m. S.R.C. serum.

Examination of splenic blood taken 4½ hours after passing the first sample of urine.

No malarial parasites.

Pigment (simple tertian) in endothelial cells and mononuclear leucocytes.

Phagocytosis of altered and apparently normal red blood corpuscles a very conspicuous feature. Small cells with crescentic nuclei containing each a single often quite normal looking blood corpuscle are abundant. These cells have but little protoplasm and what there is is extended around the engulfed cell.

In some of the cells the protoplasm shows some fine red granules. The cells vary somewhat in size, the larger ones approaching in appearance mononuclear leucocytes, but the most numerous are small and nearly globular in shape.

The included red cells are generally quite unaltered, a little paler than the free cells in the neighbourhood; others are pale with a faint yellowish tinge or show a rim of darker staining substance around the periphery. The closest scrutiny failed to show in these cells either malarial parasites or any indication of contained bodies.

Cells resembling these are seen, which in spite of the small quantity of protoplasm they possess have engulfed two, three, or even four red cells, and others of the same type are seen packed with a dozen or more red cells in various stages of decolouration.

Of macrophages there are two types:—

1. Cells of the nature of those just described which appear to be free cells and are generally more or less globular.
2. Large endothelial plaques often having two nuclei and containing besides red cells various vacuoles and included masses.

In both types of cell the red cells vary from normal to small dark cells and cells in various degrees decolourised.

Occasionally a shrunken cell of a greenish tinge is seen. A differential count of 2,200 cells in the spleen gave:—

	Per cent.
Large macrophages	5·5
Small mononuclear cells of various types	49·8
Large mononuclears leucocytes	4·4
Polymorphonuclear leucocytes	13·7
Eosinophile leucocytes	·7
Large cells with basophilic protoplasm	1·8
Cells of doubtful nature, free nuclei, etc.	24·0
	99·9
Large macrophages containing red cells	1·7
Small mononuclear cells containing red cells	1·3

The condition of the included red cells is shown by the following:—

Unaltered cells	Large macrophages	3
	Small mononuclear phagocytes	37
Partially altered cells	Large macrophages	10
	Small mononuclear phagocytes	9
Vacuoles derived from engulfed red cells.	Large macrophages	22
	Small mononuclear phagocytes	5

Small mononuclear cells which appear to be of the same type as the small phagocytes already noted were numerous. They appeared very frequently to have been in active amœboid movement when fixed by the drying of the film.

Blood—

Count of red cells gave on different dates—

	15th January 1908.	19th January 1908.	22nd January 1908.
	Per cent.	Per cent.	Per cent.
Polychromatic cells	*Nil*	2	18

	16th January 1908. Per cent.	19th January 1908. Per cent.	22nd January 1908. Per cent.
Large corpuscles	46	34	23
Medium corpuscles	52	40	53
Small corpuscles	2	24	4

Counts of 500 leucocytes gave :—

	16th January 1908. Per cent.	17th January 1908. Per cent.	18th January 1908. Per cent.	19th January 1908. Per cent.	22nd January 1908. Per cent.
Polymorphonuclears .	63·7	61·7	57·4	49·4	73·
Large mononuclears .	23	28·7	22	25·5	9
Small mononuclears .	9·3	9·5	14	19·8	9
Eosinophiles . .	1·8	2·1	1·6	3	5
Macrophages . .	2	...	5	.	3

Urine.—Estimation of amount of hæmoglobin.

Time.	Specific gravity.	Albumen grammes per litre.	Urine Quantity.	Percentage Hæmoglobin.	Total Hæmoglobin (blood equivalent).
				Per cent.	
2-30 P.M., 16th January 1908 . . .	...	1·05	60 c.c.	10	6 c.c.
11 P.M. „ . . .	1018	·6	750 c.c.	6·6	50 c.c.
9 A.M., 17th January 1908 . . .	1017	·6	550 c.c.	6·6	37 c.c.
7 P.M. „	1018	·7	550 c.c.	6·6	37 c.c.
12 P.M. „	1020	·7	350 c.c.	6·6	23 c.c.
12 NOON, 18th January 1908 . . .	1018	·5	450 c.c.	5	22·5 c.c.
12-50 P.M. „ . . .	1020	·3	300 c.c.	2·5	8 c.c.
				TOTAL .	183·5 c.c.

Urobilin shown by zinc test.—Zinc urobilin bands visible on ten-fold dilution in all the samples.

Urine deposit—

2-30 p.m. 16th January 1908.—Casts not noticeable. Deposit chiefly formed of minute globular masses.

11 p.m., 16th January 1908.—Deposit similar to last but contains a small number of epithelial cells.

9 a.m., 17th January 1908.—Deposit contains many cells. Cells with double nuclei common. Cells appear to be kidney epithelium.

7 p. m., 17th January 1908.—Deposit largely composed of such cells and cell *débris.*

12 noon, 18th January 1908.—Many cells and granular urates.

Phosphates.—Much increased in latter samples.

Case XXI.—Ellenbari. February 1908.

European. Age—35.

Length of residence in Duars.—5 years.

History.—Was at Faskowa, Eastern Duars. Went home; returned in November 1907. Has taken quinine since coming out on Mondays and Fridays. Says he was previously quite fit, but was noticed by his medical adviser to have been looking latterly "very seedy." He states himself that he has had a heavy cold which has made him feel weak in his legs (temperature ?).

21st February 1908.—Late at night took his ordinary dose of quinine.

22nd February 1908, 4 a.m.—Had a rigor and passed hæmoglobinous urine.

Seen by us at 10 A.M. on the same day. Spleen several inches below costal margin. Complains of severe epigastric pain. Yellow tint just noticeable.

Blood.—Serum of an intense yellow colour with orange tint. Blood received in 5 per cent. citrate and centrifugalised showed hæmoglobinæmia.

Isotonic point.—First definite increased tint at ·425. Marked hæmolysis at ·4 per cent. salt.

Hæmosozic value.—1·258 per cent.

Films taken at 10 A.M., 4 P.M. and 9 P.M. showed no parasites or pigmented leucocytes.

A count of 500 leucocytes gave :—

Polymorphonuclears	73·6 per cent.
Large mononuclears	20·2 ,,
Small mononuclears	6· ,,
Eosinophiles	·2 ,,

Urine.—Hæmoglobinous urine passed all day.

23rd February 1908.—Urine hæmoglobinous up to 10 A.M. Spleen just below costal margin. Marked yellow colouration of the conjunctivæ and skin. Anæmia much more pronounced than yesterday.

24th February 1908.—Patient convalescent. Temperature 99. Spleen just palpable. Urine free from hæmoglobin.

25th February 1905.—At night had a rise of temperature to 103.

27th February 1908.—Given 2½ grains of quinine at 9-30 A.M., 1-30 P.M. and 5-30 P.M.

28th February 1908.—Given 2½ grains of quinine at 2 A.M., 6 A.M., 10-30 A.M. and 2-30 P.M.

5 P.M., slight rigor.

5-15 P.M., passed 8 oz. hæmoglobinous urine.

Hæmoglobinuria continued till 10 P.M. There was a second rigor at 6-40 P.M. Urine at 1 A.M. next morning free from hæmoglobin, but temperature remained between 101 and 103 throughout the whole of the 29th and showed an evening rise to 100 on the following two days.

Estimation of amount of hæmoglobin in urine—

Time.	Urine Quantity.	Percentage hæmoglobin.	Total hæmoglobin (blood equivalent.)
		Per cent.	
22nd February 1908, 4 A.M.	312 c.c.	2	6·25 c.c.
„ 7 A.M. to 8-30 A.M.	625 c.c.	3	18·75 c.c.
„ 10 A.M.	284 c.c.	5	14·2 c.c.
„ 1 P.M. to 2 P.M.	570 c.c.	5	28·5 c.c.
„ 3 P.M. to 4-30 P.M.	510 c.c.	3	15·3 c.c.
„ 6 P.M.	285 c.c.	3	8·5 c.c.
„ 7 P.M. to 9-30 P.M.	655 c.c.	3	19·6 c.c.
„ 10-30 P.M. to 23rd February 1908, 6 A.M.	510 c.c.	3	15·3 c.c.
„ 7 A.M.	310 c.c.	2	6·2 c.c.
„ 9 A.M.	300 c.c.	·5	1 c.c.
Total	...	...	133·6 c.c.

Case XXII.—Baradighi. April 1908.

Marwari. Age 18.

Native country.—Hissar district, Punjab.

Length of residence in Duars.—9 months.

History.—Suffered ever since coming to the Duars from repeated attacks of fever. States that he had fever two or three times every month. In December this man was seen by us and found to have enlarged spleen and quartan parasites in his blood. Another man in the same house had a simple tertian infection.

Takes rice, dhal, chappattie, and butter, never fish or meat.

In his own country cannot remember feling ill.

About a fortnight ago being feverish took 20 grains quinine in two days. Ten days later he got high fever and was given diaphoretic mixture. Next day in the morning when his temperature was down was given 10 grains quinine sulphate.

23rd April 1908.—Just afternoon had high fever again with rigor, headache, and a temperature of 104. At this time passed reddish black urine.

24th April 1908.—Passed four specimens of reddish black urine.

Seen by us about 6 P.M. on this day.—Pallor marked. Yawning. Spleen below umbilicus. Splenic puncture performed shortly after seeing patient. Film showed scanty crescents, pigmented endothelial and mononuclear cells, also large macrophages with engulfed red cells and many included nucleated cells resembling very small mononuclear leucocytes.

Washed B.W.F. corpuscles.	*Normal serum.*	*Hæmolysis.*
½ c.c. Washed B.W.F. corpuscles emulsion.	(150 c.m. normal serum + 50 c.m. B.W.F. serum at 37° for 15 m.)	No hæmolysis. Agglutination.
1 c.c. Washed corpuscles native.	150 c.m. B.W.F. serum.	No hæmolysis. Agglutination.
½ c.c. Washed corpuscles very anæmic native.	150 c.m. B.W.F. serum.	Agglutination.

25th April 1908.—Temperature fallen to 96. Urine free from hæmoglobin.

Case XXIII.—Baradighi. April 1908.

Coolie. Age 58.

Native country.—Ranchi, Chota Nagpur.

Length of residence in Duars.—14 years.

History.—Unknown.

2nd April 1908.— Had fever with temperature of 104.

3rd April 1908.—Temperature 104. Vomiting. Passed reddish black urine.

4th April 1908.—Continued to pass black urine. Jaundice.

5th April 1908.—Temperature in the morning 102. Urine a little clearer.

6th April 1908.—Temperature in the morning 99, in the evening 102.

Seen by us on this day.—Marked anæmia. Slight jaundice. Hæmoglobinuria ceased. Films of peripheral blood showed some engulfed red cells, shadows, endothelial plaques, polychromasia, and uncleated red cells. No parasites or pigmented leucocytes.

Case XXIV.—Huldibari. May 1908.

Chinaman. Age 35.

Native country.—China.

Length of residence in Duars.—3 years.

History.—States has had much fever at first but not so much latterly. Had two days fever recently.

7th May 1908.—Seen by Dr. Babu who found he had a temperature of 105° and was passing hæmoglobinous urine. Temperature in the evening 103.

8th and 9th May 1908.—Temperature still high.

Seen by us next day.—Was passing very small quantities of very dark, nearly black hæmoglobinurious urine. Anæmia. Marked yellow colouration of conjunctivæ. Spleen slightly enlarged. Splenic puncture performed. Spleen blood showed much recent malignant tertian pigment, both scattered and in the form of reliquats, also a segmenting malignant tertian parasite.

Blood.—Serum showed orange tint characteristic of hæmoglobinæmia. No tendency for clot to dissolve in serum. Blood received into citrate solution shows hæmoglobinæmia.

Films of peripheral blood show some large endothelial plaques and some included red cell shadows.

A count of 500 cells gave:—

Polymorphonuclears	75
Large mononuclears	11·7
Small mononuclears	12·3
Transitional	·5
Eosinophiles	·3

Films of splenic blood showed distinct agglutination and many spherocytes. Some decolourised included red cells present. Macrophages contain engulfed small mononuclear cells.

Hæmolysis experiments.

½ c.c. washed B.W.F. corpuscles emulsions.	150 c.m. normal serum.	Rapid hæmolysis.
½ c.c. washed B.W.F. corpuscle mulsions.	150 c.m. normal serum + 50 c.m. B.W.F. serum at 37 for 15 min.	No hæmolysis. Agglutination.
Washed B.W.F. corpuscles	Allowed to stand in ·9 per cent. salt.	No hæmolysis.

11th May 1908.—Small quantity passed free from hæmoglobin. Hæmoglobinæmia no longer to be detected. Serum bright yellow but free from hæmoglobin.

Case XXV.—Nagasuri. May 1908.

Bengali Babu. Age 30.

Native country.—Murshidabad.

Length of residence in Duars—5 years.

History.—At his home scarcely had one attack of fever in the year; in the Duars had two or three attacks every month. Has been one year at Nagasuri living in the midst of coolie lines.

29th May 1908.—About midnight he had an attack of fever but with a more severe sensation of cold and shivering than usual. In the morning noticed that his urine was red.

30th and 31st May and 1st June 1908.—Passed hæmoglobinous urine. Had severe epigastric pain and much vomiting, especially on the 31st. Jaundice was first noticed on the 30th and was very marked on the 31st.

2nd June 1908.—Urine dark in the morning but subsequently cleared. Blood film taken by Dr. McCutcheon.

Seen by us on the 3rd.—Hæmoglobinuria over. Spleen enlarged. Yellow colouration of conjunctivæ present. Marked tenderness over the liver, less so over the spleen. Serum yellow. No hæmoglobinæmia. Clot dissolved to some extent overnight though it did not do so in a control blood.

Hæmolysis experiments.

½ c.c. washed B.W.F. emulsions + 150 c.m. normal serum, S.R.C.	No hæmolysis.
	No agglutination.
Washed B.W.F. corpuscles + Undiluted normal serum, S.R.C.	No hæmolysis.
	No agglutination.

Case XXVI.—Indong. July 1908.

Bengali Babu. Age 25.

Native country.—Dacca.

Length of residence in Duars.—3 years.

History.—Previously lived in Sylhet where he did not have any serious fever. Since coming to the Duars has had fever not less than once a fortnight and has been home for this season. Had a previous attack of Black-water Fever at Bogra. Has been one year at Indong.

5th July 1908.—Noon.—Felt indisposed and took 10 grains quinine.

3 P.M.—Rigor.

4 P.M.—Passed high coloured urine, and noticed at the end of micturition some drops of blood coloured fluid.

7 P.M.—Passing hæmoglobinous urine.

10 P.M.—Seen by Dr. McCutcheon who noted his temperature to be 105, and took blood films.

Seen by us 4 P.M. 6th July 1908.—Temperature 103. Passing dark red hæmoglobinous urine. Yellow colouration distinct. Spleen 3 fingers below costal margin. Splenic puncture performed.

Blood.—Serum orange yellow. Blood received into citrate shows small amount of hæmoglobinæmia. No solution of clot in the serum.

Isotonic point.—5 P.M. 6th July 1908.

Percentage salt	·525	·5	·475	·45	·425	·4
	tint.	tint.	tint.	tint.	tint.	increased tint.
Control	*nil.*	*nil.*	*nil*	*nil.*	tr	h

due to hæmoglobinæmia.

Hæmosozic value . . . . . 1·175 per cent.

Splenic blood received into hypertonic salt solution gave only same tint (approximately) as peripheral blood. Stained with neutral red in 3 per cent. citrate solution showed many dark staining corpuscles, more than were present in peripheral blood. Films showed large numbers of macrophages of mononuclear type containing numerous red cell shadows. Phagocytosis of red cells are present. Agglutination very noticeable. Spherocytes present. Malignant tertian pigment scattered and in the form of reliquats.

A count gave :—

Large shadow corpuscles	1	per cent.
Small poikilocytic shadows	·05	,,
Spherocytes	·4	,,

Hæmolysis Experiments—*Vide* Part IV, Chapter II.

Patient's urine +	Emuls. patient's corps.	No hæmolysis. No agglutination.
150 c.m. urine +	150 c.m. ditto. plus 50 c.m. normal serum.	No hæmolysis.

8th July 1908.—Hæmoglobinuria still present up to late in the day.

Isotonic point.—

Percentage salt .	·476	·45	·425	·4
		nil.	tr	tr
Control . .		tr	h	inc H

Serum contains no recognisable hæmoglobin but is of a strong yellow colour.

Case XXVII.—Monabari. July 1908.

Bengali Babu. Age 48.

Native country.—Dacca.

Length of residence in Duars.—18 years.

History.—Until coming to Monabari 18 months ago did not suffer much from fever, but at Monabari has suffered constantly and severely. Had a previous attack of Black-water Fever (Case XI) in October 1907. A week before present attack was feeling feverish.

9th July 1908.—6 A.M.—Took quinine 5 grains.

10 A.M.—Took another 5 grains.

2 P.M.—Passed hæmoglobinous urine. Temperature in the evening 105.

10th July 1908.—Temperature in the morning 100. Passed very dark urine all day.

Seen by us at 4 P.M. 10th July 1908.—Passing hæmoglobinous urine. Temperature 104. Spleen enlarged. Yellow colouration.

Blood.—Serum rosy red. Hæmoglobinæmia as shown by adding blood to citrate solution and centrifuging equal in amount to that shown in the serum. Estimation of amount of hæmoglobin showed this to be about equivalent to 3·75 per cent. solution of blood.

Isotonic point—

Percentage salt .	·476	·454	·434	·416	·4	·384
			incr	incr		
	tint	tint	tint	tint	inc H	
Control . .	*nil.*	*nil.*	*nil.*	h	hh	inc H

Hæmosozic value— 1·07 per cent. salt.

Blood.—In solution of neutral red in 3 per cent. citrate showed some dark staining corpuscles. A count gave 5 in 20 fields.

Hæmolysis Experiment.—*Vide* Part IV, Chapter II, page 150.

Urine.—An estimation of the amount of hæmoglobin gave:—

1st sample passed	10	per cent.	blood equivalent.
Urine passed 10th July 1908 . .	1	„	„
Hæmosozic value of urine . .	1·28	„	salt.

11th July 1908.—Hæmoglobinuria still continues. Temperature in the morning 102. Serum ruddy yellow tint. Estimation of the amount of hæmoglobinæmia gives about 1·5 per cent. blood solution.

Isotonic point—

Percentage salt . .	·476	·454	·434	·416	·4	·384
	tint	tint	tint	incr tint	inc H	
Control . . .	*nil*	*nil*	*nil*	*nil*	hh	inc H

12th July 1908.—Hæmoglobinuria over.

13th July 1908.—Given quinine 5 grains.

14th July 1908.—Given quinine 5 grains.

15th July 1908.—No quinine administered.

16th July 1908.—A relapse. Stated to have come on apart from administration of quinine.

17th July 1908.—Urine dark red. Temperature in the morning 105, in the evening 104.

18th July 1908.—Temperature 103 in the morning, 101 in the evening. Hæmoglobinuria ceased towards evening.

Case XXVIII—

Mr. M.—European Assistant. Aged about 29.

Length of residence in the Duars.—Between four and five years all on one garden.

Arrived in Duars.—December 1903.

History.—Had a mild attack of Black-water Fever in May 1905. Used to get a good deal of fever and did not take quinine regularly, only when fever occurred, and then not for long. Has had a lot of fever since March 1908, and in the middle of May 1908 had a mild attack of intermittent Black-water Fever lasting two days off and on. On the 5th June went for a sea trip to Rangoon. While on board ship had two relapses very slightly. Was not then taking quinine. The first attack in May 1908 came on after taking quinine hydrochloride. Got back to the garden on June 15th and was feeling very fit. On the 17th had a bit of fever and took 10 grs. quinine hydrochloride and remained at work. Fever came on again on the next day at 5 P.M., 9 P.M. took grs. 5 quinine hydrochloride, and during the night at 3-30 A.M. another grs. 5. Next morning took grs. 5 again at 7 A.M., and another tabloid a little later on the garden. Went out to work. After coming in at midday passed black urine at 2 P.M. but went out again into the garden for a short time. Then came in and went to bed. Woke up at 9 P.M. shivering. Got hot bottles and finally began to sweat and bilious vomiting became troublesome.

Notes.—5th day, June 23rd.—When patient was first seen he was found to be lying ill in a bungalow near the tea house, within 20 yards of a line harbouring servants and tea house men and their families. Altogether nearly sixty souls, including over 30 young childrren. Enquiry showed that quite a number of these people were recent arrivals, having been but two months or so in the Duars. Among some thirty examined all had enlarged spleen and ten had fever, while all but two showed malarial parasites in the blood. Alongside this line and within 80 yards of the bungalow was a small drain from the tea house in which many thousand

larvæ of Barbirostris and other anopheles were discovered. In the bungalow many specimens of M. Listoni, Barbirostris and other species were seen.

On examination patient was found to be sallow and slightly jaundiced, liver not enlarged but spleen nearly 4 fingers breadth below ribs and rather tender. The urine which had remained brackish-red for over four days was commencing to clear but still showed bands of oxy and met-hæmoglobin. Patient was markedly anæmic, hæmoglobin standing at 30 per cent. Temperature was about 100.

June 24th.—Patient remained fairly well until about 10-20 when the temperature rose to 101, and shortly after he passed clear urine.

June 25th.—Patient remained well until 9-20, when he had a slight rigor, and shortly after passed dark red urine containing oxyhæmoglobin, while his temperature rose to 102·5, dropped again with a slight sweat to 100, but immediately afterwards rose once more to 103.

Temperature fell towards evening and urine cleared slightly.

June 26th.—At 9-2 P.M. temperature rose again, reaching 102·5 at 12 A.M., but though the urine was a little dark in colour it was nearly free from hæmoglobin. Temperature dropped again that evening, the urine also improving.

June 27th.—The morning temperature was only 99, but at 8-20 there was a rigor, and black urine was passed, while the temperature speedily rose to 103·3, dropping after a short interval to 102·6, only to rise again to 104. By this time the urine had become quite clear again. That evening as the temperature appeared to be typically malignant tertian in character, although microscopic examination failed to show parasites, grs. 5 quinine bi-hydrocloride was administered hypodermically.

June 28th.—Morning temperature was 99, but it rose to 102 at midday, though urine remained quite clear. Towards the evening temperature dropped.

June 29th.—Morning temperature normal, but at 11 A.M. it rose to 100, falling a little later to nearly 99, but again rising to 101. Urine remained clear, and patient was given another 5 grs. of quinine.

June 30th.—Morning temperature normal, but gradually rising after midday to 102 in the evening. Quinine grs. 5 was administered. Urine remained quite clear and temperature fell during the night.

July 1st.—Temperature in the morning normal. Patient was given grs. 5 quinine at day-break. At about 10 A.M. there was a rigor, and a little later black urine was passed, while the temperature rose to 103, fell again to 102·5, and again rose to 104·2.

At evening it had again fallen to 99, and the urine was quite clear once more. From this time the quinine was stopped again entirely.

July 2nd and 3rd.—Urine remained quite clear and though temperature rose each day to 101 or 102 for a short time, the patient appeared to be doing well.

July 4th.—Patient's morning temperature 99·2, urine quite normal. No quinine had been given since the grs. 5 on the morning of the 1st. At 1-30 P.M. there was a sharp rigor, temperature rose to nearly 103, and once more black water was passed. By 4 P.M. the urine was again clear, and temperature fell rapidly after 5 P.M. to nearly normal.

July 5th.—From this morning quinine was once more administered, as it was evident that unless something could be done to check the disease the patient would die before he could be removed. 1½ grs. were given at intervals totalling 7½ grs. per diem. There was a little smoky urine at 11 A.M. on this day, but nothing further, the treatment being continued during the ensuing week, temperature each day remaining more nearly at the normal, and the urine keeping quite clear, while the spleen became smaller in size. Finally patient became sufficiently well to be sent to England.

The most interesting features of this case are the number of relapses within a short period; the facts that the attacks followed hydrochloride of quinine in the first case; that subsequently a relapse occurred on six occasions without any relation to quinine administration, and that on three occasions such a relation could be traced. A number of blood specimens were taken which have not yet been fully examined.

(*Dr. R. L. Paterson's case included to show association of Black-water Fever with quinine hydrocloride and calcium chloride.*)

Case XXIX.—Tezpur, Assam. Attacked with Black-water Fever—

(1) April 19th. (2) July 22nd. (3) August 4th.

V. F.—Tea planter, aged 24, European.

Length of residence in Assam.—15 months.

History.—Arrived in Assam from England January 1907. Kept good health a short time and then had severe attack of malaria (with malignant tertian parasites in blood). Subsequently took quinine as a preventive, but somewhat irregularly. Had further attacks of fever necessitating a short trip on account of health. Kept fairly fit during cold weather of 1907-08.

Attack.—(1)—*April 19th.*—Felt "rotten" but worked. Took quinine, grs. 10, in the evening. After midnight had a rigor, vomited, fever came on together with diarrhœa. Urine not seen.

April 20th.—Passed typical black-water at 11 A.M., temperature 104. Jaundice present. Blood showed no parasites. At 3 P.M. urine sherry coloured. Recovery rapid. Went a trip to Ceylon, and while there got fever. Took quinine hydrochloride grs. 15, with no ill-effects. Returned to work and remained fit, taking quinine hydrochloride, grs. 10, regularly once a week.

Attack.—(2) *July 22.*—Felt feverish. Took quinine hydrochloride, grs. 10, in the forenoon, but remained at work. At 7 P.M. took another grs. 10 quinine hydrochloride and went to bed. Vomited soon after. At midnight had a rigor and vomiting. At 3 A.M., 5 A.M. and 6 A.M. passed black urine, total of 26 ozs.

July 23.—Temperature 103·4 all day. Blood showed no parasites. Jaundice present and urine still black, 26 oz. being passed during the day and 22 oz. at night.

July 24th.—Temperature normal. Urine still black, but clearing a little towards evening. Spleen and liver not enlarged or tender. Total urine passed 17 ozs.

July 25th.—Urine became clear, amber in colour; no blood pigment or albumen. Quantity only 11½ ozs.

July 26th to 31st.—Steady improvement.

August 1st.—Headache. Temperature 104. Blood examination showed numerous small ring malarial parasites. Urine clear. Was given calcium choloride, grs. 25, preparatory to quinine treatment. Temperature fell slightly.

August 2nd.—Urine clear. Was given quinine hydrochloride grains 5. Temperature rose to 103·8. Was given another grs. 5 quinine and temperature fell Urine remained clear. Calcium chloride continued, in grs. 10 doses.

August 3rd.—Urine remained clear. Blood showed no parasites. Temperature 100 to 101. Quinine hydrochloride grs. 5 given morning, midday and evening (total grs. 15). Urine still clear.

Relapse, August 4th.—After midnight on the 3rd had a rigor and vomiting. Temperature rose to 104·2. At 4 A.M. passed 6½ ozs. black urine. Temperature fell to 100, but urine remained black all day and jaundice was present. Total urine 33 ozs. Calcium chloride continued. During night passed 25 ozs. of urine.

August 5th.—At 6 A.M. urine cleared slightly and at 10 A.M. was sherry coloured. It contained no blood pigment or albumen. Blood showed no parasites. Patient rapidly became convalescent, and left for England on the 7th.

Case XXX.—Tezpur, Assam. Attacked Black-water Fever, May and June 1901.

E. P., Tea-planter, aged 42, European.

Length of residence in Assam.—Uncertain, but had passed considerable time in Cachar. Had also been in China. About four years in district where attack occurred.

History.—Had suffered a good deal from malaria, having been obliged to go away for over a month previous November. Had enlarged spleen. About beginning of May began to get attacks of malaria, simulating acute gastritis. Vomiting and retching occurred every second day. Fever so slight as to be overlooked by patient. About middle of May went to another garden on a visit. While there attacked with severe fever. Took much phenacetin and some quinine.

Attack.—(1) Passed black and deep red urine for two days, and became somewhat jaundiced. When a little better went for a trip, but while on the river had at return of fever. Took grs. 20 of phenacetin, and suffered from collapse. Fever continued. Blood examination showed small ring malarial parasites.

Attack.—(2) Urine bloudy in colour. Patient would not take quinine on account of nausea and vomiting. Euchinin obtained and administered in grs. 10 doses (30 grs. before noon each day, at which time temperature used to rise). Urine cleared after two days, but fever continued longer, although parasites were not found. Convalescence complicated with dysentery. Patient recovered and went to England.

Case XXXI.—Tezpur, Assam. Attack Black-water Fever, October 1905.

A. B. T., Tea-planter, aged 35, European.

Length of residence in Assam.—About 13 years.

Length of residence in Tezpur.—About 7 years.

History.—Had suffered occasionally from severe malaria, once nearly dying from an attack which may have been Black-water Fever. Went to England on leave in 1903 and returned in the cold weather. Kept fairly fit during 1904, but got attacks of fever and biliousness rather often during the rains of 1905. Had rather a severe attack of fever towards the end of September, but did not remain from work in consequence. Only took quinine when attacked with fever. Early in October had a severe attack of fever, but still attempted to remain at work. Bilious vomiting was a marked feature of this attack which was supposed to be largely "liver." On October 6th took a purgative, and for the fever several doses of phenacetin followed by quinine. That night had a rigor with vomiting and passed dark urine. Got up next day, but vomiting (bilious) and fever continued. Took more phenacetin and quinine. When seen was found to be jaundiced, with a high temperature, a tender and enlarged spleen, and a tendency to retch and vomit bilious matter. Temperature about 103. Blood examination negative. Urine very dark reddish black but in large amount. On boiling a deep brown deposit came down half filling the test tube. After 48 hours the urine commenced to clear the spleen rapidly reduced in size, and the jaundice largely disappeared, but a marked bronzing of the skin remained and was still present some months later.

Case XXXII.—Tezpur, Assam. Attacked, December 1905.

A. B. H., Tea-planter, aged 25, European.

Length of residence in Assam.—Seven years.

History.—Came to Assam in 1898. Suffered very often from malaria. Had an enlarged spleen for several years and on one occasion passed dark urine which however was not seen by others. In 1904 changed his bungalow, going to live in one wihtin a short distance of a populous coolie line. While here had attacks of fever at frequent intervals. Used to take quinine very irregularly, 20 to 30 grs. one day because of fever, and then no more for a few days until again feeling "seedy." Had been suffering at intervals of about 10 to 14 days for several months before Black-water Fever occurred. On several of these occasions blood examination showed malignant tertian infection. Ten days before the Black-water Fever patient had a typical, attack of tertian fever (36 hours' fever, a remission of 12 hours and again fever). Malignant tertian parasites were present in the blood. Quinine was taken as before, but was shortly afterwards stopped, owing to its being "forgotten."

Attack.—Day before attack had a return of fever, and took a dose of quinine and went to work, and next day felt rather better. In the morning, however, took 20 grs. of quinine and went to work, but complained of feeling ill, and passed highly coloured urine. In the evening was still at office work but felt

cold, and when seen was looking sallow and haggard. Advised to go to bed, but while undressing had a rigor. About an hour after passed dark red urine. Temperature rose but only to 99, slight nausea and bilious vomiting, with tenderness of the spleen and pain at the pit of the stomach. Blood examination showed no parasites. Urine on boiling showed a deposit of albumen half filling test-tube. Next morning considerable jaundice observed. Great weakness present, patient being unable to sit up or even to turn over without assistance. Towards the evening the urine commenced to clear and by next day was apparently normal. Temperature also came to normal very quickly, but the spleen remained enlarged and tender for some time. Convalescence was fairly rapid, but was marked by a great tendency to suffer from indigestion. Nine months after had slight attack of jaundice, but no hæmoglobinuria.

Case XXXIV.—

Babu J. N. N., native shopkeeper, aged 25.

Belongs to Burdwan in Bengal, but has resided in the Mateli bazar for the past four years.

History.—Has had a good deal of fever. Nine months ago was away in his country for five months. Since his return has often had slight fever and twice severe attacks.

July 8.—Was feeling all right.

July 9.—Had fever in the evening, but took no quinine.

July 10.—Had fever, but no treatment.

July 11.—Had fever, but no treatment.

July 12.—Had still got fever and becoming frightened, took 3 doses of grs. 5 each quinine. At 7-30 noticed dark urine and temperature rose from 103 to 104·6 in the night.

July 13.—When seen urine was commencing to clear. There was marked bilious vomiting, vomit being of a dark green colour. Jaundice was marked, and the spleen was enlarged a hand's breadth below ribs. There was not much tenderness of the epigastrium. The serum was intensely yellow coloured and on examination with the microspectroscope showed absorption bands similar to urobilin. No hæmoglobin could be detected in the serum at this time. Blood examination showed no parasites on casual examination, complete examination has not yet been possible. Blood corpuscles gave an isotonic point of ·425 per cent. salt solution, and gave an isolysin reaction, hæmolysing with class A serum. The serum did not hæmolyse class A corpuscles.

RECORD OF CASES OF BLACK-WATER FEVER IN THE DUARS.

*Dam Dim District.**

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Barons	1	Mr. C.—Assistant. Age 28.	May 1889 .	Sept. 1889 .	Came out November 1887. Both attacks mild. Went home shortly after second attack. Recovery.
	2	Mr. E.—Assistant. Age 25.	Oct. 1891 .	Mar. 1892 .	Was out about 3 years. Twelve months at Baitagool, then went to Barons. Second attack had 3 days' hæmoglobinuria, then 2 days' high fever without hæmoglobinuria or anæmia and this was followed by death.
	3	Mr. M.—Assistant. Age 28.	Aug. 1890.	...	Out 3 or 4 years in Sylhet, then came to Barons. Death.
	4	Mr. D.—Assistant. Age 28.	Oct. 1899	Feb. 1901	Out about 5 years. One year at Narkati. Two years Rungamuttee, then Barons. First attack went home for 6 months. After coming back had second attack, then went Sylhet.
	5	Mr. G.—Assistant. Age 25.	May 1901 .	...	Was out about 3 years. Kumlai 18 months, then to Barons. Remained at Barons some years, but did not have any further attack. Severe attack. Recovery.
	6	Mrs. C— . .	May 1895 .	Sept. 1896 .	Out 2 years. States had fever every week for the whole period. Second attack at place near Darjeeling. Recovery.
	7	Mr. T.—Assistant. Age 23.	June 1907 .	...	Out 8 months, all at Barons. After first few weeks in the country suffered from repeated and severe fever. Went later to Assam and had second attack there. Was sent home. Severe attack.
	8	Babu H. Age 25 .	July 1902 .	...	Two years at Barons from country. Recovery.
	9	Babu S. G. . .	Feb 1904 .	...	About 1 year resident. Came from his country with wife and daughter. Lived at North Grant. Mild attack. Recovery.
	10	Babu S. G.'s wife.	Feb. 1904 .	...	Lived at North Grant. Death.

* NOTE.—Information mainly contributed by Dr. Brown of the Dam Dim District.

Dam Dim District—contd.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Barons.	11	Babu S. G.'s daughter. Age 8.	Sept. 1903 .	Dec. 1904 .	Lived at North Grant. Death.
	12	Babu N. D. G. .	...	...	Eight or 9 years in Duars. Went to visit country, had Black-water Fever after coming to Barons.
Sissibari	13	Mr. W.—Assistant. Age 26.	Oct. 1901 .	...	Out 2 years, all at Sissibari. Two days' continuous hæmoglobinuria with polyuria. Death.
	14	Mr. F.—Assistant. Age 26.	Sept. 1906 .	Dec. 1906 .	Had been out in the Chelsa District some years. Went home and came out to Sissibari. Attacked 18 months after. Left district 6 months after. Second attack. First attack severe. Second attack mild, lasting few hours. Recovery.
	15	Mr. T.— Age 35 .	Jan. 1889 .	...	Called "Yellow Fever."
	16	Babu. Age 28.	...	...	Eight years in Duars. Short time only at Sissibari. Hæmoglobinuria lasted 2 days, clearing on the 3rd.
Baintbari	17	Mr. C.—Assistant. Age 26.	Nov. 1889 .	...	Out 2 years, all at Baintbari. Attack was severe, lasting about 2 days. Recovery.
Bullabari	18	Mr. S.—Assistant. Age 28.	Jan. 1897 .	...	Two years in Duars. Born and lived previously in Darjeeling. Had a relapse in both attacks without administration of quinine. Patient being in charge of a nurse. First attack lasted 2½ days: there was an interval of 1 day, then hæmoglobinuria for 2 days, but not so severe as at first. Second attack lasted about the same time. After second attack had fever for about 4 days. At this time had 2½ grs. quinine every three hours subcutaneously, but no Black-water Fever ensued.
	19	Babu's cook . .	Oct. 1905 .	...	.....
	20	Babu . . .	Feb. 1907 .	...	.....
	21	Mr. G.— Manager. Age 40.	...	...	Out 17 years. Barons 9 months. Rungamuttee 5 years. Neora Nuddy 1 year. Rest of the time at Bullabari. Went home about 2 years before attack for 8 months. Attack lasted 2½ days, but amount of hæmoglobinuria small. Mild attack. Recovery.

Dam Dim District—contd.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Bullabari	22	Servant . .	Unknown .	Aug. 1907 . .	First attack mild. Second attack seen by us after death.
	23	Mrs. G. . .	July 1896 .	...	6 months in Duars. Suffered greatly from fever, but quinine not taken on account of pregnancy.
	24	Mr. S.—Assistant. Age 28.	Jan. 1897 .	...	2 years in Duars; all the time at Bullabari. Death.
Ranacherra	25	McK.—Child. Age 3 years.	Aug. 1905 .	...	Attack lasted about 18 hours. Hæmoglobinuria slight. Born at home; came out age of 3 months. Mild. Recovery.
	26	Chinaman—Case IX.	Sept. 1906 .	Oct. 1907 .	.....
Rungamuttee	27	Chinaman . .	Dec. 1904 .	...	Attack lasted about 2 days. Recovery.
	28	Chinaman . .	Unknown .	...	Attack lasted about 2 days. Recovery.
	29	Chinaman . .	Unknown .	...	Attack lasted about 2 days. Recovery.
	30	Babu's son. Age 18.	Apl. 1894 .	...	Had been in the district about 2 years. Attack lasted 1½ days.
	31	Barber . .	July 1907 .	...	In the district 12 or 15 years Constant change of garden Suffers now from tingling in hands and feet with attacks of numbness.
Meenglas	32	Mr. W.—Assistant. Age 22.	Dec. 1904 .	Oct. 1905 .	Out 2½ years; all the time at Meenglas. First attack lasted only 6 hours. Second attack severe, lasting 3 days. Recovered from hæmoglobinuria, but death took place after subsequent fever which occurred 2 days after attack and lasted 4 days. Interval of 2 days normal temperature. Death.
	33	Mr. D.—Manager. Age 34.	Sept. 1906 .	...	8 years; out all the time at Meenglas in same bungalow. Went home 2 years previously. Suppression. Death.
	34	Babu's servant—Second attack—Case III—*Vide* Bullabari.			

Dam Dim District—contd.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Meenglas	35	Mussalman bearer —Case VI.	Aug. 1907 .	...	Attack over in less than 24 hours.
	36	Babu—Case V .	Aug. 1907 .	...	20 years in the Duars.
Pathajora	37	Mr. H.—Manager. Age 45.	Apl. 1899 .	...	Had been out 1 year, then went home for a few months and had an attack 8 months after return. Attack lasted 2 days. Death from suppression. Severe case. Death.
	38	Malpaharia Sardar —Case XII.	Apl. 1899 .	...	15 years on garden.
Hahiapathia	39	Dr. Babu . .	Unknown .	Unknown .	8 years in the district. Had 3 previous attacks. Fourth in November 1905.
	40	Babu . . .	Jan. 1908 .	...	5 years in Duars. At Upper Chengmarie, then 10 months at Hahiapathia.
Toonbarri	41	Mr. M.—Manager. Age 27.	Dec. 1887 .	...	Out 3 or 4 years, about 2 years at Toonbarri. Attack lasted about 3 days. Severe. Recovery.
	42	Babu's daughter. Age 9.	June 1907 .	...	.....
Baithagool (abandoned).	43	Mr. McB.—Assistant. Age 23.	May 1888 .	Oct. 1889 .	Came out in 1887. Barons. Rungamuttee, 6 months; 2 or 3 weeks only at Baithagool. Attack lasted 2 days.
Sungachi	44	Mr. W.—Assistant. Age 22.	July 1905 .	Nov. 1905 .	Out 18 months; all the time at Sungachi. Second attack lasting 8 hours, occurred during recovery from typhoid. Temperature rose to 106 and polyuria followed. Recovery.
Bagracote	45	Mr. D.—Assistant. Age 25.	Jan. 1900 .	...	Out 2 years; all at Bagracote. Actual attack occurred at Jalpaiguri. Lasted 2 days.
	46	Babu Bhose .	Oct. 1901 .	...	Attack lasted 1½ days.
	47	Dr. Babu—Case VIII.	Sept. 1907 .	...	Chalouni 5 years; went to his country for several months and on returning went to Bagracote. Attack 2 or 3 months after coming to garden.
Washabari	48	Khitmatgar .	Oct. 1899 .	...	Was given hypodermic quinine when urine was very black; next morning urine cleared up.

Dam Dim District—contd.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Neora Nuddy	49	Mr. P.—Manager	Sept. 1897 .	...	Frenchman. In the district 8 months; came from Pondicherry. Attack lasted 18 hours. Recovery.
	50	Mr. L.—Age 32 .	Oct. 1905 .	Dec. 1906 .	Out 10 years. Left Sungachi, December 1904; went for 6 months to recruiting district. (Santal Parganas). Took charge at Neora Nuddy April 1905. Both attacks lasted 12 hours. Mild. Recovery.
	51	Mr. S.—Manager. Age 38.	Jan. 1907 .	...	Out 10 years. Went to Neora December 1901. Went home and came out end of 1902; stayed at Neora and had fever, 1905; went to Sungachi for 12 months and had very healthy year. Beginning of 1906 went again to Neora and had repeated fever coming at shorter and shorter intervals till Black-water Fever supervened. Attack lasted 6 hours. Mild. Recovery.
	52	Head Babu .	Nov. 1904 .	...	Out 18 months or 2 years. Went afterwards to Hahiapathia. Recovery.
	53	Lachman Nagpuri (tea man).	...	...	Stated to have had 2 or 3 mild attacks.
Glencoe	54	Mrs. B.— . .	July 1907 .	...	Out 1 year 9 months; all the time at Glencoe. Attack lasted 2 days. It actually occurred at Jalpaiguri. Severe. Death.
	55	Mr. R.—Assistant. Age 26.	June 1900 .	...	Severe. Death.
Manabari	56	Mr. R.—Assistant. Age 28.	Dec 1903 .	Nov. 1904 and Aug. 1905.	3 years out when first attacked; all the time at Manabari. First attack severe. Third attack Mild. Recovery.
	57	Dr. Babu—Case XI; Case XXVII.	Oct. 190 .	July 1908 .	...
Ghora Bhutan	58	Mr. T.—Padre. Age 36.	Dec. 1906 .	...	Had lived in Darjeeling Terai some time. Went home; came to Duars and was attacked 1 year after arrival. Attack lasted 3 days. Ghora Bhutan only head-quarters; spent much time travelling in Duars. Severe. Death.

Dam Dim District—concld.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Needam Jhora	59	Babu . .	Feb. 1904 .	...	Attack lasted 3 days. Severe. Death.
Ellenbari	60	Mr. S.—Manager. Age 40. Case XXI.	Feb. 1908 .	...	Was at Paskowa since 1903. Went home 1905 and came out in December 1905 and went to Ellenbarri December 1907. Attack lasted 2 days with relapse following 5 days after first attack ended. Severe. Recovery.
Baintguri	61	Mohammedan bearer Age 32. Case XIX.	Dec. 1907 .	...	12 years in Duars. Came to Baintguri 5 months ago and was living at Chota bungalow until 6 weeks before attack. Attack lasted about 12 hours. Mild. Recovery.
Phoolbari	62	Mr. D.— . .	October 1895	...	About 2 years in the Duars.
	63	Mr. F.— . .	...	...	.....
Gheish River	64	Negro (half-caste). Age 35.	...	...	Working on railway 4 or 5 years in district.
Latiguri	65	Daughter of above. Age 8.	...	...	Came out to father about 2 years before attack.

*Chelsa District.**

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Nagasuri	66	Mr. G.—Assistant. Age 22.	July 1898 .	...	2 years in the country; all at Nagasuri Chota bungalow. Had frequent almost daily attacks of fever. Recognised by his companions as unusually liable to attacks. Onset of hæmoglobinuria sudden, red urine being the first symptom. Duration 80 hours. Severe. Recovery.
	67	C.—Child. Age 6	Nov. 1898 .	...	6 months in the Duars. Bara bungalow. Hæmoglobinuria not intense. Slight. Recovery.
	68	Head Babu. Age 39.	June 1902 .	...	Had been at Nagasuri since 1893. Hæmoglobinuria 48 hours. Severe. Recovery.

* NOTE.—Information contributed by Dr. McCutcheon, Chelsa District.

Chelsa District—contd.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Nagasuri	69	Bhutia woman .	Dec. 1902 .	...	Living in lines at Chota bungalow suffering from fever for a week before attack. Severe. Death.
	70	Dr. Babu's wife .	Sept. 1906 .	...	Passing backwards and forwards to country. Hæmoglobinuria 56 hours. Recovery.
	71	Babu . .	Oct. 1907 .	...	Fifteen months from Cooch Behar Hæmoglobinuria transient. Slight. Recovery.
	72	Babu—Case XXV	May 1908 .	...	Four years in country. Last year in Nagasuri. Hæmoglobinuria lasted 3 days.
Chalouni	73	Babu. Age 25 .	Sept. 1898 .	...	On the garden for 3 or 4 years. Attack of ordinary fever 3 days before. Hæmoglobinuria ceased second day.
	74	Dhobie . .	Oct. 1900 .	...	Hæmoglobinuria lasted 3 days.
	75	Dhobie . .	Oct. 1907 .	...	Hæmoglobinuria about 24 hours.
Engo	76	Eurasian. Age 30	Sept. 1898 .	June 1900 .	Five years at Engo. Hæmoglobinuria lasted about 24 hours. Recovery. Second attack at Tissati. Death.
Chelsa	77	Mr. T.—Assistant. Age 24.	June 1898 .	1900 . .	Three years in the country. At Sam Sing there about end of 1898 to Chelsa Chota bungalow (recovery). Second attack on way home. Death.
	78	Mr. D.—Assistant Age 25.	...	Sept. 1901 .	Four or 5 years in Duars. Previously at Bagracote. (*Vide* No. 45).
Sathkaya	79	Miss C.—Age 22	Sept. 1900 .	...	Second year in India; all the time at Sathkaya. Used to get attacks of "bilious fever." Living at Bara bungalow.
Kilkott	80	Dr. Babu . .	Oct. 1901 .	1903 and Jan. 1907.	Two or 3 years in the country. Hæmoglobinuria remitted a number of times. Recovery.
Moortee	81	Babu . .	May 1907 .	...	Six or 8 years in the country. Hæmoglobinuria lasted less than 24 hours.
	82	Dhobie . .	Dec. 1906 .	...	Slight and transient hæmoglobinuria.
Mytelie	83	Mr. T.—Assistant. Age 24.	Aug. 1899 .	...	In second year. Chota bungalow. Used to get occasional slight fever. Hæmoglobinuria lasted 2 days. Recovery.

Chelsa District—concld.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
Baradighi 84	Babu. Age 30. Case XVIII.	Dec. 1907 .	...	Nine years in the country. Two or 3 months at Baradighi. From home 15 months before. Hæmoglobinuria lasted about 12 hours.
85	Babu. Age 28. Case XVII.	Dec. 1907 .	...	Eight years in the country; last returned from leave, December 1906. Hæmoglobinuria lasted about 12 hours with slight relapse. Had been 6 weeks only at Baradighi.

Nagrakata District.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attacks.
Looksan 86	Mr. C.—Assistant. Age 23.	Nov. 1906 .	...	Resident in Duars 2½ years, 3 months at place of attack. Had malaria and "liver" from time to time, and states that he had a mild attack of Black-water Fever 14 months previously. Before this last attack had had two attacks of fever, subsequent to which he had not taken any quinine, until November 10, when he took grs. 10. On the morning of the 12th he passed black urine. He was found to be jaundiced, with spleen and liver enlarged and tender, and pain over the loins. At one time temperature rose to 108, but fever ceased on 5th day. A remarkable feature of this case was the occurrence of double amblyopia, from which patient subsequently recovered.
Chengmarie 87	Babu. Age 23	July 1907 .	Nov. 1907 .	Had been 3 years in Duars, only 6 months at Chengmarie, and at Neora Nuddy previously. Had fever two days before attack and took quinine. When seen admits having had fever every month for past 3 years. Temperature rose to 105, jaundice marked and spleen enlarged 3 fingers below rib. No parasites were found in the blood. It was noticeable that three severe rigors occurred before onset of dark urine, which was gradual. Had fever several times afterwards and two days before second attack.

Nagrakata District—contd.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
Nagrakata 88	European child. Age 5.	Oct. 1907 .	...	Had passed all her life in the Duars. Had three days' fever and passed black urine after quinine on the 4th day. A very transient attack with a slight relapse on the 3rd and 6th day afterwards. Blood examined on the 14th day (after attack) showed quartan parasites. Spleen was considerably enlarged and restlessness was a marked feature. Recovered.
Chengmarie 89	Uriah mali. Age 35.	July 1907 .	...	Had been 3 years in Duars: 1½ years at Ghatia and 1½ years at Chengmarie. Occasional attacks of fever and spleen 3 fingers' breadth below ribs. On July 6th got fever, but had no treatment as he lived alone. Passed very dark urine on the 27th. On the 28th had grs. 5 calomel but no quinine. Became comatose on the 30th. Liver enlarged, spleen very tender. Suppression took place and death ensued. *Post-mortem* showed evidence of recent malaria in the organs; more fully described among our own cases.
Bhamandanga 90	Mr. W.—Assistant	Aug. 1903 .	...	Had only been out in the country about 8 months when Black-water fever occurred and he died.
Hilla 91	Babu . . .	Aug. 1901 .	...	Had previously had what may have been a slight attack of Black-water Fever. Death. Was in Assam in 1900.
Ghatia 92	Babu . . .	Aug. 1901 .	...	Length of residence in Duars not known. Death.
Ghatia 93	Mrs. C. . .	1904 .	...	Particulars not yet to hand.
Nagrakata 94	Mr. B. . .	July 1896 .	...	Had previously had an attack while at Kolabari. Had been at Nagrakata since the end of 1892, but had changed bungalows and had lived at the Bara bungalow for 18 months. States that he kept good health and was very fit. In March 1905 was away for a sea trip. Used to get attacks of headache coming on suddenly and lasting half a day. Black-water Fever came on after a sharp attack of fever which began 2 days before

The above cases are extracted from notes furnished by Dr. Stone of Looksan, Nagrakata, Duars.

Nagrakata District—concld.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
					the Black-water Fever. Had not taken quinine regularly, only occasionally when fever occurred. Recovery.
Nagrakata	95	Mr. O. . .	Dec. 190	1902 .	Had previous attack of Black-water Fever in 1896. Went home on leave in 1898 returning at the end of the year. Used to get a good deal of fever and "liver." Had three attacks of Black-water Fever in six years, but since quinine grs. 5 has been taken daily; has had no further attack during past six years.
Carron	96	Mr. M.—Assistant	Oct. 1891 .	...	Had been 3 years 7 months in the Duars, distributed as follows: 2½ years at Chalouni, where he got a lot of fever, and 16 months at Carron. Didn't get much high fever here, but a lot of biliousness and vomiting and yearly crops of boils. Never took quinine regularly and for fever not more than 10—15 grs. in 24 hours. Black-water Fever followed a ten day bout of low fever with biliousness, and lasted 5 days. The doctor came on the 3rd day, and after bringing temperature down to 100 with grs. 15 antipyrin gave a dose of grs. 30 quinine, followed by 20 grs. doses every 4 hours. Next day reduced dose to grs. 10. Made an uneventful recovery.

Dino-Torsa District.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Telepara	97	Dr. S.— . .	Nov. 1894 .	...	Had been out in Duars only two years. Attack lasted 3 days.
	98	Dr. N.— . .	...	...	Had more than one attack; no particulars yet received.
	99	Dr. H. W.— .	...	...	Particulars not yet to hand.
	100	Dr. D.— . .	...	...	Three attacks; all mild; dates not yet furnished.
	101	Mr. F.—Planter .	...	...	Had two attacks on this garden and one at Bandipani.
	102	Babu J.—Head Clerk.	Feb. 1899 .	Mar. 1900; Apl. 1901.	Went to Telepara in 1898 and had Black-water Fever following year. Used to get a great deal of fever each year for 4 years. Last attack occurred in the Jalpaiguri station.

Dino-Torsa District—contd.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Telepara	103	Babu S.—Tea House Babu.	...	...	No particulars received.
	104	Babu D—Clerk .	...	...	No particulars yet to hand.
	105	Chinaman . .	...	...	No particulars received.
	106	Mr. P.—Assistant	...	Nov. 1906 .	Had two or more attacks. Had only been out about 2 years. Death.
Dim Dima	107	Mr. M. . .	June 1898 .	...	Died from Black-water Fever after four days' illness.
	108	Mr. S. . .	Jan. 1898 .	July 1899 .	Had been 6 years in India, 4 years in Cachar and 2 in the Duars. Had a lot of fever after coming to Duars, and for nearly 3 weeks before Black-water Fever had been having nearly continuous low fever. Did not take quinine regularly. Urine dark for 2 days and jaundice present. Went for a trip to Ceylon and had a slight relapse in the Hooghly. Second attack on board ship going home. Previously much low fever. Urine dark for 4 days, and jaundice marked. Severe kidney pain felt and attack followed by 20 to 30 boils on the face. Recovery.
Dim Dima	109	Mr. M.—Assistant	Sept. 1901 .	...	No further particulars.
Birpara	110	Mr. G. . .	Oct. 1897 .	...	Had been home for 6 months. He did not take quinine. Death.
Beneguri	111	Mr. M.—Assistant	Feb. 1907 .	...	Had previously been at Dim Dima for six months. Attack came on very suddenly. Recovery.
Hantapara	112	Mr. K.—Assistant	Jan. 1906 .	...	Had been in Duars nearly 4 years. Used to take quinine frequently in an irregular manner. Died of heart failure.
Lankapara	113	Chinaman, carpenter. Age 34.	Apl. 1906 .	...	Had been 10—12 years in Duars, but on and off for 2-3 years in Hantapara. Had occasional attacks of fever. Took quinine for fever only. Recovery.
	114	Paharia boy. Age 6.	Sept. 1906 .	...	A very mild case, the boy not willing to lie up. Recovery.

Dino-Torsa District—concld.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Bandipani	115	Mr. E. . .	Oct. 1897 .	October 1898.	Had a third attack some time later on another garden.
	116	Mr. F. . .	Oct. 1898 .	...	Had had two previous attacks at Telepara. Died after five days' illness. Had been in the country a number of years.
Tasati	117	Mr. M.—Assistant	May 1905 .	May 1908; July 1908.	First attack very mild. Second attack also very mild, followed by a very severe attack with many relapses in June, fuller particulars of which are given among our own cases. Recovery.
	118	Eurasian . .	June 1900 .	...	This patient had previously suffered from Black-water Fever at Engo in Chalsa District. Death.
Kolabari	119	Mr. B.—Assistant	Sept. 1892 .	.	Had previously been three years in Sylhet, and while there had a good deal of fever and one illness which may have been Black-water Fever. Was at Huldibari 2 years and got a lot of fever. Had been only 3 months at Kolabari and got a lot of fever. Went to Darjeeling.
		Mr. T.—Assistant	June 1896 .	...	Came out from England, January 1895, and was only out 18 months when he died of Black-water Fever.
Huldibari	120	Chinaman, carpenter.	May 1908 .	...	Fully reported among our own cases. Patient recovered from Black-water Fever, but died shortly afterwards from severe hæmorrhage from the stomach.
Hurtalguri	121	One case . .	...	...	No particulars yet to hand.

Torsa District.

Name of garden.		Name.	1st attack.	2nd attack.	Particulars of attack.
Chuapara	122 .	Mr. P.—Planter. Age 30.	July 1897	...	Had been 8 to 10 years in India, but only 2 years in place of attack. He had gone home on leave 3 years before attack. Had suffered much from malaria and took large doses of quinine, but attack did not follow quinine.

Torsa District—contd.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
Hasimara 123 .	Mr. T.—Planter. Age 30.	Aug. 1898 .	...	Had been 8 to 10 years in India and about 4 years in place of attack. He had been home on leave from February to May previous to the Black-Water Fever. He was supposed to be strong and healthy, and not to suffer much from fever, but he took quinine occasionally and in very large doses. Death.
124	Mr. G.—Assistant	Sept. 1908 .	...	Had been 4 years in Ceylon and 3 in Calcutta, and came to the Duars in February 1908. Symptoms pronounced. Jaundice, dark port-coloured, urine becoming nearly solid on boiling. Bilious vomiting frequent. Before attack he appeared to be in good health and not to suffer from fever, but he was very careless. He frequently took quinine. Recovery.
Notes of the above three cases contributed by Dr. H. A. Knyvett-Hoff, Rungamuttee, Central Duars.				
Hasimara 125	Mr. D.—Planter .	Octr. 1903	Very mild attack	while travelling in the district.

Jainti District.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
Rydak 126 .	Mr. F.— .	June 1896 .	July 1897 .	Came to India 1904 when aged 21, and went to Sylhet. Had constant attacks of fever. Early in 1895 went to Duars and had much malaria. Fever continued to be frequent in 1896 and Black-water Fever occurred. During 1897 had constant attacks of fever until Black-water Fever again occurred. Then left the district. Later attacks of Black-water Fever see Dam Dim list.
Newlands 127 .	Mr. M.—Assistant	Sept. 1902 .	Sept. 1903 .	Patient was then nearly two years in the country. Had been getting fever and ague some time before attack. Had taken large doses of quinine at intervals of two or three days. Second attack more severe. Had not had "fever" until three days before attack. Had taken no quinine since last attack which had been supposed

Jainti District—contd.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
				to be quinine poisoning. Black-water lasted 4 days. The situation of the bungalow was bad, drainage difficult and coolie lines near. Recovered.
Rydak 128 .	Mrs. S.—Age 35	Feb. 1907 .	...	Had an attack of Black-water Fever on the way home after leaving Rydak. Had been in the Duars nearly 3 years. Had spleen and slightly enlarged liver and was anæmic, but had not complained much of fever before the attack of Black-water Fever. Case also given by Elwyn Jones.

RECORD OF CASES OF BLACK-WATER FEVER IN ASSAM.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
Tezpur District 1	Dr. M.— . .	Aug. (?) 1897.	...	Had been in Assam less than a year. Lived on a very malarious garden and had suffered much from fever. Severe case with suppression of urine. Death.
2	Mr. O.—Assistant	1897 (?) .	...	Had been in Assam about two years or less. Very severe case. Death.
3	Mr. P.—Manager. Age 42.	May 1901 .	June 1901 .	Many years in Cachar, Assam, also in China. Suffered much from malaria in garden upon which his Black-water Fever occurred. Had been resident there nearly 4 years. Severe attack with one or more relapses. Recovery.
4	Babu Garden doctor. Aged 33.	June 1903 .	...	Had been some 12 years in Assam and the Duars. Had been just under 2 years on the garden where attack took place. Lived within 20 yards of large coolie line with an endemic index of 98 per cent. During his attack enormous acute enlargement of both liver and spleen occurred, which subsided 3 days after the hæmoglobinuria ceased. Recovery.
5	Babu Garden clerk	Sept (?) 1903	...	Was living on a garden that was intensely malarious, and had suffered much from fever. Very severe attack. Death.
6	Mr. T.—Manager	Oct. 1905 .	...	Had returned from leave to England just about two years. Had suffered considerably from malaria, especially for some time prior to attack. Severe case. Recovery.
7	Chinaman . .	Nov. 1905 .	...	Resident on same garden as last case. Had been there under 2 years. Very severe case with suppression. Death
8	Mr. H.— Age 25.	Dec. 1905 .	...	Had been out about 7 years, but had changed gardens three times in this period. Had suffered frequently from attacks of malignant tertian malaria, and had an enlarged spleen at the time of attack.

Name of garden	Name.	1st attack.	2nd attack.	Particulars of attack.
Tezpur District 9	Madrasee cook	Dec. 1906	...	Had been resident for less than a year on a very malarious garden. Got a lot of fever, but would not take any quinine. Had taken no quinine at all previous to attack of Black-water Fever. Severe case. Recovery.
10	Babu Garden clerk	Dec. 1906	...	No particulars known as to length of residence, etc. Severe case with suppression. Death.
11	German Missionary.	Feb. 1907	...	Had been less than a year resident in a native village. Was very cachectic and weak when found with Black-water Fever. It is very doubtful if any quinine had been taken in this case prior to attack. Severe attack. Death.
12	Mr. T.—Manager. Age 32.	Dec. 1906	...	Had been some five years in the Duars, and then had gone to Assam. Was in England on leave during 1905. After his return had had a lot of malaria for more than a year. Did not take quinine regularly, only when fever occurred and then only in small quantities. When Black-water Fever occurred had only been 7 days in Tezpur. Previous two years spent in Upper Assam, at Margharita. Day previous to Black-water Fever had fever and took a 5-gr. tabloid of quinine. Attack which was of the greatest severity was remarkable for being nearly afebrile, temperature only rising to 103 first day and then going to normal until death.
	Notes of case contributed by Dr. Drake, Hatibari. T. E., Tezpur, Assam.			
13	Mr. F.—Assistant. Age 24.	April 1908	July 1908; Aug. 1908.	Had been in Assam less than 2 years. Had had a good deal of fever in spite of very irregular quinine prophylaxis. The case, fuller notes of which are given on a previous page, is important on account of Black-water Fever occurring after quinine hydrochloride and calcium chloride.
	Notes kindly sent by Dr. R. L. Patterson.			

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
Nowgong District. 14	Mr. S.—Planter. Age 26.	Mar. 1880 .	...	About 1 year in Nowgong. Had been out 8 years, but went home from Behar, returning to India 1878. Had a great deal of fever and took quinine in very large doses. Had taken a big dose at dinner, night of attack. Did not get jaundiced. Recovered.
15	Mr. M.—Planter .	1879 rains .	...	Had been out many years in Upper Assam, and had not long returned from leave to England. Had an attack of high fever, jaundice, restlessness, black-coloured vomiting, etc. Death.
16	Mr. F.—Assistant	1897 . .	...	Only 15 months in Assam. Had a lot of fever, and lived near coolie lines. Was never really fit. When Black-water Fever occurred was treated with heroic doses of quinine—120 grs. in 12 hours. Very severe case, but recovered and went home with enlarged spleen and liver.
17	Mr. D.—Planter .	Oct. 1904 .	...	Ten years in Assam, but had been to England on leave in 1902, returning at the end of 1902. Had been "seedy" on and off, especially for the 6 weeks before the Black-water Fever. Took quinine grains 15 at noon and had Black-water Fever at 4 P.M. Lived near coolie lines. Recovered.
18	Mr. N.—Assistant	Aug. 1907 .	...	Had been out in Assam 6 years and had kept apparently pretty fit. Lived near coolie lines. Had some few days malaise and then took 35 grains of quinine in less than 24 hours, together with calomel 10 grs. and 1 oz. magnesium sulph. Very severe case. Black-water Fever lasting nearly 4 days. Recovered.
	Cases Nos. 16, 17	and 18 from	notes sent by	Dr. Dodds Price of Nowgong.
Tura, Garo Hills. 19	American child. Age 12.	Feb. 1887 .	...	Went to America when 6 years. Returned to Assam when past 9. Had travelled about in the hills with his father. Was found to be ill, and had some quinine given to him. This was followed by Black-water Fever, which occurred on 3 or 4 occasions. Recovered.

Name of garden.	Name.	1st attack.	2nd attack.	Particulars of attack.
Tura, Garo Hills. 20	American child. Age 4.	Apl - May 1899.	...	Child had been a few weeks in Darjeeling, and had two mild attacks of Black-water Fever. Subsequently he had several other attacks after May 1899. They appeared after small doses of quinine. Temperature sometimes went to 107.
	Came to Assam when 4 months old. Had no less than 8 attacks of Black-water Fever.	June 1900—July 1900.	...	In June 1900 he had euchinin in 1 gr. doses without ill effect, but later he had fever every second day (tertian), and after a dose of 4 grs. of euchinin severe Black-water Fever occurred and after 40 hours resulted in death.
21	American child. Age 9.	Aug. or Sept. 1900.	...	Had also been in Darjeeling with the boy, and had shown similar symptoms of Black-water Fever after taking quinine. She had gone to America when 2½ years old, returning to Assam when 5 years. Recovered.
Lakhimpur District. 22	Babu B. C., Native Doctor. Age 34.	Nov. 1907 .	...	A native of Dacca, nearly 10 years on garden where Black-water Fever occurred. Had severe malaria in 1901 for some 6 months. Went on short leave to country in 1905. Had been taking quinine 10 grs. once a fortnight for some time before attack.
	Notes	contributed by	Dr. Falkiner,	Assam.
23	Mr. G.—Planter. Age 45.	Mar 1907 .	...	Had been many years in Assam, and had been to Burma. Went to England on leave in 1904 or 1905. Returned to Assam and went to Burma again doing much travelling and camping. Much run down before attack, and looked sallow and ill. Took grs. 10 of quinine. Black-water Fever followed and lasted 3 days. Great epigastric pain. No spleen or liver felt. Recovered.
	Notes contributed	by Dr. Mc Combie, Assam.		
Jorhat District, Assam. 24	Mr. F.—Assistant. Age 23½.	Oct. 1907 .	...	Had been 3 years in Assam, and 5½ months on garden where the attack occurred. Previously had been in Nowgong. Had had occasional attacks of fever. Took quinine as a preventive, but doses or times not stated. Black-water Fever followed quinine administation. Recovery.

Name of garden.		Name.	...st attack.	... attack.	Particulars of attack.
North Lakhimpur District. Assam	25	Mr.—Planter .	1905 . .	...	No particulars received. Death.
	26	Mr.—Planter .	1906 . .	...	No particulars yet received. Death.
Cachar	27	Mr.—Railwayman	1905 . .	...	An engine driver who had been at one time on the Gold Coast. Death.
	28	Babu — Postmaster.	1900 . .	...	This followed quinine. Recovery.
Sylhet	29	Mr. J.—Assistant. Age 25.	1901 . .	...	Had come out from England at the beginning of 1899. Was living on an unhealthy garden near the hills. Severe attack of Black-water Fever followed period of many malarial attacks in 1901. Recovered 1901.
	30	Mr. T.	Sept. 1907 .	...	Patient had been less than a year in India and had already had a previous attack in the Duars. Recovered.
	31	Mr. A.—Manager	June 1908 .	...	Had previously been 3 or 4 years in Assam, and was in his third year in Sylhet. Death.
Bishnath District, Assam.	32	Miss—Nurse .	1903 or 1904	...	Had not been very long in Assam; full particulars not yet to hand.

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