NOTES ON SIXTEEN CASES OF BLACKWATER FEVER OCCURRING IN MALTA.

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The following notes are founded on sixteen cases of blackwater fever which occurred during the months October, 1916, to April, 1917 (inclusive). The patients were soldiers who had been invalided during the preceding five months from Macedonia, where malaria and blackwater fever are endemic, to hospitals at Malta, where blackwater fever was not known to have occurred and where malarial infection does not take place owing to the absence of anopheles. Fifteen of the cases were first attacks, the sixteenth was a second attack of blackwater fever occurring in Malta three months after the first, which occurred in the malarial district above mentioned.

We are personally responsible for the greater part of the laboratory examinations and are indebted to the medical officers in charge of the cases for permission to make use of the clinical notes.

Since the causation and pathology are at present uncertain it is necessary to define blackwater fever as a clinical syndrome.

Essential conditions are that the patient must have resided in a malarial district for at least some months and probably have had repeated attacks of malaria, these conditions were fulfilled in all our cases.

The symptoms which are invariably present are hæmoglobinuria, fever, and a high degree of anæmia, appearing very early and increasing rapidly during the attack, and prostration. Some degree of jaundice and vomiting are almost constant symptoms and in our cases were only absent in Case 16, which was a mild second attack. Enlargement and tenderness of the spleen and liver are commonly present and often increase during the attack. There may be a considerable degree of abdominal pain, generally referred to the epigastrium or hypogastrium.

It is possible that two or three distinct diseases are grouped under the name of blackwater fever. However, a perusal of the cases recorded by Christophers and Bentley [1] and by Barratt and Yorke [2], and in the British Government reports on blackwater fever in tropical Africa for 1912 [3] and 1913 [4], leads us to the conclusion that the cases occurring in the tropics cannot be differentiated clinically from those which we have observed in Malta.

TABLE I.

No. of case	Name	1st or 2nd attack of Blackwater fever	Date of onset of hæmoglobinuria	Interval between onset of Blackwater fever and: Arrival in malarial district	First attack of malaria	Arrival in non-malarial country	History of quinine treatment before onset of Blackwater fever	Species of malaria parasite	Date when parasite found	Examination for parasites during attack	Result
				Months	Months	Months					
1	Me. ..	1st	10.10.16	—	1·6 (50 days)	1·4 (42 days)	Last 17 days, quin. sulph., gr. x; often vomited; 9.10.16, quin. hydro., gr. x	P.f.R.	8.9.16 and 9.9.16	Negative	R.
2	Ja. ..	1st	23.12.16	12	5	1	Quinine continuously since onset of malaria	?	?	Negative	R.
3	C. ..	1st	25.12.16	11	5	2	?	?	?	Negative	R.
4	T. ..	1st	23.1.17	15	5	4	?	P.f.	Aug., 1916	Negative	R.
5	K. ..	1st	24.1.17	11	4	3	15.1.17 to 21.1.17, none; 22.1.17, quin. sulph., gr. xxx; 23.1.17, quin. sulph., gr. lx; 24.1.17, quin. sulph., gr. lx	P.f.C.	22.1.17	Negative	R.
6	Ma. ..	1st	10.2.17	—	5·5	2	4.12.16 to 3.1.17, quin. sulph., gr. xxx; 4.1.17 to 17.1.17, quin. sulph., gr. xx; 18.1.17 to 25.1.17, quin. sulph., gr. x; 26.1.17 to 9.2.17, quin. sulph., gr. xxx	P.f.R.C.	6.1.17	Negative	R.
7	R. ..	1st	11.2.17	16	4	3	Quinine continuously since September, 1916; 1.2.17 to 9.2.17, quin. sulph., gr. x; 10.2.17 and 11.2.17, quin. sulph., gr. xv	P.f.R.C.	31.1.17; 1.2.17; 7.2.17; 1.4.17	Negative	R.
8	He. ..	1st	13.2.17	8	4	3	20.1.17 to 11.2.17, none; 12.2.17 and 13.2.17, quin. sulph., gr. v	P.f.R.	21.1.17	Negative	R.
9	S. ..	1st	27.2.17	18	5·75 (or 6 years)	5·5	10.2.17 to 24.2.17, none; 24.2.17 to 27.2.17 (incl.), quin. sulph., gr. xxx	P.f.R.C.	27.2.17	Positive, 27.2.17, P.f.R.C.	R.
10	Hay...	1st	2.3.17	15	5·5	2	5.2.17 to 23.2.17, quin. hydro., gr. xx; 24.2.17 and 25.2.17, none; 26.2.17 to 1.3.17, quin. hydro., gr. xx	P.f.R.	18.1.17; 25.2.17; 2.3.17; 3.3.17	Positive, 2.3.17 and 3.3.17	R.
11	G. ..	1st	7.3.17	?	4·5	3·3	From (?) to 28.2.17, quin. sulph., gr. xv; 1.3.17, none; 2.3.17 to 4.3.17, quin. bih., gr. xv i.m., and quin. sulph., gr. xv p.o.; 5.3.17 and 6.3.17, quin. sulph., gr. xxx; 7.3.17, quin. bih., gr. xv, i.m., and quin. sulph., gr. xv p.o.	P.v. and P.f.R.	1.3.17; 2.3.17; 3.3.17; 4.3.17, P.v.; 13.11.16, P.f.R.	Negative	D., 14.3.17
12	W. ..	1st	22.3.17	?	8	5	Quinine since July, 1916	P.f.R.	22.3.17	Positive, 22.3.17, P.f.R.	D., 27.3.17
13	Jo. ..	1st	7.3.17	7·5	5·5	2	2.1.17 to 7.3.17, quin. gr. x to gr. xxx	P.f.R.	21.1.17	Negative	R.
14	D. ..	1st	1.4.17	16	5·5 (or 10 years)	4·5	Quinine regularly; latterly one dose daily till onset	P.v.	28.4.17	Negative	R.
15	Y. ..	1st	4.4.17	?	8·5	4	Quinine, none for 1 week immediately before onset	?	—	Negative	D., 8.4.17
16	Had...	1st	18.1.17	7	5	0	?	P.v.	?	?	—
		2nd	14.4.17	—	—	1·75	Quinine sulph. for 6 weeks, gr. v; 12.4.17 and 13.4.17, quin. sulph., gr. xxx	—	—	Negative	R.

TABLE II.

No. Name Age	Onset of illness	First observation of hæmoglobinuria	Duration of hæmoglobinuria. Quantity in 24 hours	Fever	Jaundice	Vomiting	Pain	Spleen	Liver	Hæmoglobin and blood corpuscles in blood	Complications	Quinine treatment and saline	Result
(1) Me. 22	9.10.16, T. 103° F.; ill, vomiting	10.10.16, 4 p.m., porter-coloured	12 hours 20 oz. in 12 hours	3 days, T. 103°—100° F.	+ 2 days or more	+++ 2 to 3 days	+ Abdomen	++	..		..	9.10.16, quin. hydr. gr. xxx, p.o.; 10.10.16, 8 p.m., gr. x i.m. Rectal saline, 4-hourly	R.
(2) Ja. 21	23.12.16, early morning rigor, T. 103° F.; diarrhœa	23.12.16, 10 a.m., "café noir"	2½ days 40 oz. to 70 oz.	13 days, T. 104·5°—99·8° F.	++ 1 week or more	+++ 3 days	++ Hypogastrium	+++	—	23.12.17, hgb. 16%, r.b.c. 0·9 M.	..	23.12.16., quin. bih. gr. x, i.m. ,, ,, sulph. gr. x p.o. 28.12.16—4.1.17, quin. bih. gr. xv, i.m. 24.12.16, noon, saline 30 oz., i.v.; 9 p.m. 60 oz., i.v. 26.12.13, rectal saline 8 oz., b. d.	R.
(3) C. 25	24.12.16, rigor, T. 101° F.	25.12.16, afternoon very dark, porter-coloured	4 days 73 oz. to 42 oz.	5 days, T. 103·3°—99·8° F.	++ 9 days	+++ 6 days	Hypogastrium	+	..	2.1.17, hb. 28%, r.b.c. 1 M.	..	27.12.16, quin. bih. gr. xv, i.m. 28—29.12.16, quin. bih. gr. x, i.v. 30.12.16, quin. bih. gr. xv, i.m. 27—29.12.16, rectal saline 10 oz. 28 & 29.12.16, saline 40 oz., i.v.	R.
(4) T. 22	23.1.17, T. 103°—105°F.	23.1.17, very dark, porter-coloured	3½ days 63 oz. to 56 oz.	6 days, T. 105°—100° F.	+	+++ 3 days	..	+	++ Very tender		..	23.1.17, quin. bih. gr. vii, i.m. 24.1.17 ,, ,, gr. x, i.m. 25.1.17 ,, ,, gr. x, i.v. 26.1.17 ,, ,, gr. x, i.m. 27—31.1.17, no quinine 25.1.17, saline 40 oz., i.v. ,, ,, 20 oz. rectal	R.
(5) K. 23	24.1.17, afternoon headache, T. 99° F.	24.1.17, 4 p.m., dark reddish-brown	2½ days 50 oz. to 60 oz.	3 days, T. 101°—99° F.	+ 2 to 3 days	+ 1 day	Splenic region	+	—		..	25.1.17—29.1.17, quin. bih. gr. xv, i.m. Rectal saline 20 oz., 4-hourly for 3 days	R.
(6) Ma. 30	9.2.17, 10 a.m., T. 101° F., shivering, vomiting, aching	10.2.17, afternoon, dark reddish-brown	1 day	6 days, T. 103·8°—99° F.	++ 14 days or more	+++ 3 days	Abdomen, upper part: no tenderness	+ Not tender	+ Not tender	15.2.17, hb. 32%, r.b.c. 1·362 M.	..	9.2.17, quin. sulph. gr. xxx, p.o. 10.2.17 ,, ,, gr. x, i.m. 10—13.2.17, saline 10 oz. rectal 4-hourly	R.

(7) R. 20	11.2.17, 3 p.m., slight shivering, headache; 4 p.m., vomiting; 6 p.m., T. 103° F.	11.2.17, 5 p.m., blackish-red	19 hours 8 oz.	2 days, T. 103°—99·6° F.	+	11.2.17 once; 13.2.17 once	Epi-gastrium	+	..	11.2.17 hb. 62 % b.c. 3,333 M.	..	12—17.2.17, quin. bih. gr. xv, i.m. 12.2.17, saline, rectal, 20 oz. 13.2.17 ,, ,, 4-hourly 14.2.17 ,, ,, *bis die*	R.
(8) He. 21	13.2.17, 6.30 p.m., T. 104·8° F. rigor; ill	13.2.17, 7 p.m., porter-coloured	28 hours 80 oz.	4 days, T. 105°—99·8 F.	+	+ 3 days	..	+	—	14.2.17 hb. 34 %	..	14.2.17, quin. bih. gr. xv, i.m.	R.
(9) St. 27	27.2.17, 5 a.m., vomited, T. 100° F.	27.2.17, 7 a.m., dark red	3 days 30 oz. to 66 oz.	4 days, T. 103·4°—99·8° F.	++ 3 days	+ 3 days	None	+	+	..	..	27.2.17—16.3.17, quin. bih. gr. xv, i.m. Rectal saline, 4-hourly	R.
(10) Hay. 29	2.3.17, 10 a.m., T. 104° F.	2.3.17, 3 p.m., reddish-brown; 5 p.m., "café noir"	2 days 57 oz. to 86 oz.	2 days, T. 105°—100° F. (22 days, T. 104·6°—100° F.)	++ 5 days	++ 2 days	None	+ tender	+ tender	3.3.17 hb. 26 % b.c. 1,556 M.	Cystitis	2 & 3.3.17, quin. bih. gr. xv, i.m. 4.3.17, saline 40 oz., i.v. 2 and 5.3.17, rectal saline, 4-hourly	R.
(11) G. 33	7.3.17, 3 p.m., rigor, T. 103° F.; 4 p.m., vomited	7.3.17, 6 p.m., deep red	4 days; 8.3.17 anuria; 9—13.3.17 1 oz. to 7 oz.	3 days T. 105·4°—100·4° F.	+ till death	+++ 7—13.3.17	In appendix region	+	—	13.3.17 hb. 38 %	Suppression of urine	7.3.17, quin. bih. gr. xv., i.m. ,, ,, sulph. gr. xv, p.o. 8.3.17 ,, bih. gr. xxx, i.m. 9.3.17 ,, ,, gr. xv, i.m. 10.3.17 ,, ,, gr. xxv, i.v. 11.3.17 ,, ,, gr. xx, i.v. 12.3.17 ,, ,, gr. xv, i.v. 13.3.17 ,, ,, gr. v, i.v. Rectal saline, 4-hourly; subcutaneously or i.v. saline twice daily	D. 14.3.17
(12) W. 26	22.3.17, 2 p.m., rigor; 3.30 p.m., vomited, T. 104·6° F.	22.3.17, 3 p.m. dark urine	3 days 23.3.17 42 oz.; 24.3.17 none; 25.3.17 14 oz. normal colour; 26.3.17 incontinence	6 days T. 105·8°—100·5° F.	+ till death	+ 3 days	None	—	—	23.3.17 hb. 16 %	Partial suppression of urine	22.3.17, quin. bih. gr. xv, i.m. 23.3.17 ,, ,, gr. xx, i.m. 24.3.17 ,, ,, gr. x, i.v. 25—27.3.17 ,, ,, gr. xv, i.m. Rectal saline, 4-hourly 24.3.17, saline 20 oz., i.v. 24.3.17 ,, 30 oz., i.v.	D. 27.3.17

TABLE II.—*Continued.*

No. Name Age	Onset of illness	First observation of hæmoglobinuria	Duration of hæmoglobinuria. Quantity in 24 hours	Fever	Jaundice	Vomiting	Pain	Spleen	Liver	Hæmoglobin and blood corpuscles in blood	Complications	Quinine treatment and saline	Result
(13) Jo. 23	6.3.17, 7 p.m., shivering, headache, sweating	7.3.17, 8.30 a.m., red	5 days with intermissions 24 to 46 oz.	5 days 103·6°—99·2° F.	+ 3 days	++ 2 days	Headache	++	+	6.3.17, h. 52%, r.b.c., 3,682 M.	..	7 & 8.3.17, quin. bih. gr. xv, i.m. 10.3.17, quin. hydr. gr. xx, p.o. 11.3.17, quin. bih. gr. xv, i.m. 12.3.17 ,, ,, gr. xv, i.m. Rectal saline, 6-hourly	R.
(14) D. 38	1.4.17, afternoon headache, feverish	1.4.17, afternoon, blackish red	5½ days 52 to 48 oz.	4 days 105°—102° F.	++ 4 days	+++ 2 or 3 days	None	+++	+	7.4.17, h. 24%, r.b.c., 1,476 M.	..	4.4.17., quin. bih. gr. xv, i.m. Rectal saline, 6-hourly	R.
(15) Y. 31	4.4.17, 10 a.m., shivering	4.4.17, no urine; 5.4.17, 7 p.m., dark brown	4 days 3 oz. to none	4 days 104°—107° F.	+++ 5.4.17 till death	++ retching 2 days, 5 & 6.4.17	Abdomen, especially epigastrium	—	+	..	Suppression of urine	4.4.17, quin. sulph. gr. x, p.o. 5.4.17, quin. bih. gr. xx, i.m.; 11 p.m., quin. bih. gr. xx, i.v. 6.4.17, quin. bih. gr. xx, i.v. 7.4.17 ,, ,, ,, ,, 5.4.17, rectal salines, 20 oz. 4-hourly (3 retained); saline 40 oz. i.v. 6.4.17, saline 30 oz., i.v.	D. 8.4.17
(16) Had. 21	1st attack "18.1.17" "rigor," 2nd attack 14.4.17	"18.1.17"	"1 day"	..	..	..	..	..	..	..	"Epistaxis and melæna"	..	..
		14.4.17, 8 a.m.	36 hours 2 attacks with 2 days intervals	1 day 16.4.17 100° F.	None	None	None	++ Slightly tender	+	14.4.17, h. 65%,	..	14.4.17, quin. sulph. gr. xxx, p.o. No saline	R.

Summary and Discussion of the Sixteen Cases.

The following description is based entirely on our sixteen cases unless the contrary is definitely stated.

The main features of the attack were much the same in all our cases, except in the three which ended fatally with suppression of urine, and in these there was no sufficient reason for suspecting a different disease.

Details of the most important facts which were common to all our cases are recorded in two tables. Table I deals with the history of the patient before the attack and the nature of the malarial infection. Table II gives some details of the principal symptoms and the facts relating to quinine treatment.

NOTES ON TABLES.

Table I, Cols. 5, 6, and 7.—The intervals recorded are those between (1) the arrival in a malarial country (Macedonia) and the onset of the blackwater fever attack; (2) the first attack of malaria and the onset of blackwater fever; (3) the date of arrival in a non-malarial country (Malta) and the onset of blackwater fever, i.e., the minimum incubation on the assumption that blackwater fever is due to a special infection.

Table I, Col. 6.—Cases 9 and 14 had their first malarial attacks in India six and ten years ago respectively; their first malarial attacks in Macedonia were 5·5 and 5·75 months respectively before the onset of blackwater fever.

Quinine: Quin. hydr. = quinine hydrochloride; quin. bih. = quinine bihydrochloride.

The dose entered means the amount given in twenty-four hours unless otherwise stated, e.g., gr. xxx means gr. x *ter die*.

P.o. = per os; i.m. = intramuscular; i.v. = intravenous.

Malarial Parasites: P.v. = *Plasmodium vivax;* P.f. = *Plasmodium falciparum;* R. = Rings; C. = Crescents, e.g., P.f.R. = *Plasmodium falciparum*, only rings seen, no crescents.

In Cases 4 and 16 the microscopic diagnosis was made at a hospital in Macedonia and we had no opportunity of observing parasites.

Table II.—The date in Col. 2 is that considered to be the date of onset of the blackwater fever attack as shown by general symptoms. The date in Col. 3 is that of the first occasion on which "black water" was observed by the patient or medical officer. In Case 15 the onset of suppression of urine is given as the date of onset of hæmoglobinuria.

Table II, Col. 5.—*Fever:* The temperatures stated are the maximum temperature on the day on which the fever was highest, and on that on which the fever was lowest. Temperatures of 99° F. and below are not entered.

Table II, Col. 6.—*Jaundice:* + means a distinct yellow colour of the conjunctiva and skin, such as is seen not rarely in severe relapsing malaria; ++ means a more decided jaundice, such as may occur in slight "catarrhal jaundice"; +++ means a deep yellow colour which affected all the tissues, post mortem.

Table II, Col. 7.—*Vomiting:* + means vomiting on two or three occasions; +++ means severe continual vomiting.

Table II, Col. 9.—*Spleen:* + = palpable; ++ = two or three inches below costal margin; +++ = almost or quite to level of umbilicus in left nipple line; — = not palpable, no enlargement detected.

Table II, Col. 10.—*Liver:* + = slight enlargement; ++ = felt two or three inches below costal margin in right nipple line; — = no enlargement detected.

Table II, Col. 11.—*Red Blood Corpuscles:* 0·89 M. = 890,000 per cubic millimetre; 1·5 M. = 1,500,000 per cubic millimetre, etc.

R. = recovered; D. = died.

No entry in a column means no observation recorded.

Prodromal Symptoms and Onset.

The onset of hæmoglobinuria usually appears to be sudden, though in some cases the first specimen of dark urine examined was not so dark in colour as that passed a few hours later. Commonly fever, shivering and vomiting precede by one to three hours the passage of porter-coloured urine. Sometimes there may have been similar attacks of fever and shivering on the two or three days preceding the attack and it is occasionally difficult to be sure that the attack did not really start twenty-four or forty-eight hours before the "black" urine was noticed. The prodromal fever and the fever at the actual onset closely resemble malarial attacks. Our observations agree with the remark of Christophers and Bentley, that neither the patient nor the medical man can distinguish the symptoms ushering in blackwater fever from an attack of malaria.

In three of our cases (Nos. 9, 10 and 12) the malarial parasites (*Plasmodium falciparum*, rings) were found in the blood a few hours after the first appearance of hæmoglobinuria, and in four cases, Nos. 1, 7 and 10 (*P. falciparum*) and 11 (*P. vivax*), trophozoites or schizonts were found during the fever which occurred a few days before the onset of blackwater fever. In one further case (No. 5) crescents were found two days before the attacks.

These facts illustrate the intimate association of blackwater fever with active malarial infection.

The type of malaria from which the 16 cases suffered was ascertained in 11 patients; in 5 patients no malarial parasites were found whilst they were under our observation, nor were past records of the parasites obtainable. Of the 11 which were diagnosed microscopically, in 9 *P. falciparum* was found, and in 2 *P. vivax*; for two of these diagnoses (one *P. falciparum* and one *P. vivax*) we are indebted to records forwarded from other hospitals nearer the Front.

Though no record of the finding of parasites was available in five cases, the clinical history clearly pointed to the occurrence of several attacks of malaria and in one of them pigmentation of the liver and spleen was found post-mortem.

Fever.

The temperature after rising to 102° to 105° F. at or soon after the onset of the attack usually remained high but irregular till the urine became of a normal colour, then fell rapidly and after two or three days remained normal. In a few cases a secondary or post-hæmoglobinuric fever occurred which was either associated with some complication, e.g., cystitis in Case 10, or was unaccompanied by any symptoms which suggested a superadded cause. In Case 2 a return of fever occurred two days after the first fall of temperature and lasted nine days, varying from 100° to 104° F. Both the primary and secondary fevers sometimes showed a tendency to tertian periodicity.

Anæmia.

There was usually extreme anæmia. The patient as a rule appeared blanched as after a severe hæmorrhage if the hæmoglobinuria lasted more than twenty-four hours, and the hæmoglobin in the blood fell to twenty-four per cent or even sixteen per cent. In a few cases in which the attack lasted less than twenty-four hours the percentage did not fall so low, e.g., sixty-two per cent and forty-two per cent in two such cases.

The anæmia appeared to be out of all proportion to the amount of blood lost in the urine.

Jaundice.

The jaundice observed was of very different degrees of intensity. In Case 15 the conjunctiva and skin became yellow on the day following the onset of blackwater fever, and the colour became progressively deeper till death; post mortem, all the tissues were deeply bile stained. In five other cases (Nos. 2, 3, 6, 10 and 14) the colour was like that seen in mild cases of catarrhal jaundice and distinctly exceeded that observed in Cases 1, 4, 5, 7, 8, 9, 11, 12 and 13, in which it resembled the icteric tinge of conjunctiva and skin not uncommonly seen in severe relapsing malaria. In Case 16 no trace of yellow colour was observable. The jaundice often increased during the attack and began to fade after two or three days to a week. Bile pigment was only found in the urine in one case, No. 15, but the presence of a large amount of hæmoglobin may have prevented the detection of a small amount of bile pigment by the ordinary tests (HNO_3 and iodine).

The blood serum gave a positive reaction for bile pigment during life in four cases, Nos. 7, 8, 10 and 15, out of seven examined.

Hæmoglobinuria.

The appearance of the urine was usually quite characteristic. The colour was deep brownish red or blackish brown like *café noir* or porter, and a paler deposit which sometimes occupied $\frac{1}{4}$ to $\frac{1}{6}$ of the vessel appeared after it had stood an hour or so. If the urine was alkaline the colour was a very deep brownish red or crimson, and the deposit was less in amount. The urine was often so opaque that it required dilution before a spectroscope could be used. In other cases spectroscopic examination of the undiluted urine in a test-tube showed absorption of both ends of the spectrum, leaving only the red part in which the band of methæmoglobin or of acid hæmatin was seen. Determination of the kind of *pigment* present in the urine was complicated by the fact that there was usually a mixture of pigments. Methæmoglobin or acid hæmatin was in most cases present in addition to oxyhæmoglobin. The spectra of methæmoglobin and acid hæmatin are very much alike and unless these pigments are present in fair concentration the band in the red, which is the most characteristic part of these spectra, is not seen. The spectrum of acid hæmatin may for this reason be easily overlooked in a pale brown

urine. Oxyhæmoglobin is, in part at least, rapidly converted into hæmatin in acid urine. A method for estimating the degree of hæmoglobinuria is detailed at the end of this paper.

In two specimens of urine from Case 11 no spectra of hæmoglobin, methæmoglobin or acid hæmatin could be seen, although the urine was dark brown and contained much albumin. This was apparently in part due to the turbidity of the urine which had probably been in the bladder for twenty-four hours before it was drawn off by catheter. That the colour was due to altered blood pigment (probably chiefly acid hæmatin) was shown by the appearance of a reddish-purple colour due to the formation of hæmochromogen, after the addition of strong caustic soda solution. This reaction is a useful method of demonstrating the presence of altered blood pigment, for hæmochromogen not only gives a very characteristic spectrum, but the absorption bands are much darker than the bands given by the solution of hæmoglobin or hæmatin from which it is derived. Hæmochromogen was easily obtained in blackwater fever urine with caustic soda alone without the addition of any reducing substance though occasionally the purple colour did not appear till the solution had stood twenty-four hours. The hæmochromogen (reduced alkali hæmatin) is seen at the bottom of the test-tube and the pigment in the upper part is alkali hæmatin.

The *duration* of the hæmoglobinuria varied from twelve hours to five days (average 2·9 days). The urine after being dark rapidly became normal in colour in the course of twelve hours or less, and as a rule remained free from blood pigment. On the other hand, slight temporary variations in the colour occurred and a paler specimen was succeeded in a few hours by one of a very dark colour, or the hæmoglobin completely disappeared for a few hours or a whole day, and then a relapse followed. Complete intermissions of this kind occurred in two cases, Nos. 13 and 16. When the colour of the urine had recently become normal the brown centrifuged deposit, consisting of casts and cells full of yellow granules if treated with caustic soda, gave the colour and spectrum of hæmochromogen.

The *quantity* of urine passed in twenty-four hours was often increased to 60 or 80 ounces and sometimes to over 100 ounces during the hæmoglobinuria. This was no doubt in part due to the saline injections given by the rectum or intravenously. Some cases running a favourable course passed only 36 or 40 ounces. In the three fatal cases, and in these cases only, the amount of urine was much reduced; in two cases, Nos. 11 and 15, to 2 or 3 ounces in the twenty-four hours; in the third case, No. 12, the quantity was rather larger but could not be measured owing to incontinence during the last two or three days of life.

Albumin.—In the darker specimens of urine, the coagulable protein formed a very dense cloud on acidifying and boiling, which after standing twenty-four hours often occupied quarter to half of the test tube. After

the colour of the urine had returned to normal a thick cloud was generally obtained for two or three days and a trace sometimes persisted for a week or even a fortnight.

Deposit.—In the first specimens of urine passed during a blackwater attack the deposit consisted chiefly of small yellow granules; in some cases larger yellow granules or globules measuring 1 to 4 microns (0·001 to 0·004 millimetre) in diameter, which at first sight resembled red-blood corpuscles, were also present. About the second day renal cells and casts and cell-casts crowded with granules appeared. The casts and cells were in most cases very numerous and towards the end of the attack renal cells and casts packed with large yellow globules were often seen. Cells and granules were usually found in decreasing numbers for two or three days after the urine had resumed its normal colour. The scanty urine excreted when there was suppression of urine contained no renal cells or casts as a rule. A few red-blood corpuscles were found in one specimen of urine from Case 14 and in no other case. Barratt and Yorke [2] record the occurrence of a few red-blood corpuscles in the urine of a number of blackwater fever cases. It is important that the urinary deposit should be examined as soon as possible after the urine has been passed, since red-blood corpuscles are rapidly destroyed in acid urine.

Hæmoglobinæmia.

The blood serum was examined in ten cases and in four of these, Nos. 6, 9, 14 and 15, was sufficiently coloured with hæmoglobin to give a spectrum in a tube 0·3 to 0·4 cubic millimetre in diameter. Of the other six, in which blood pigment was not found in the serum, in three, Nos. 1, 7 and 10, the blood was taken only a few hours before the hæmoglobinuria ceased, in one, No. 16, during an intermission of hæmoglobinuria, and in a third, No. 4, after it had ceased; in the remaining case, No. 8, which was a mild attack lasting twenty-eight hours, the serum was examined within a few hours of the onset but hæmoglobin was not detected. Citrated plasma also was examined in two of the above cases, Nos. 14 and 15, and hæmoglobin was found in this as well as in the serum. Plasma was also examined in Case 16 during an intermission of hæmoglobinuria and no hæmoglobin was detected. In Case 15 both the serum and the plasma were examined on the first and second day of the attack and both were a deep brownish crimson on each occasion. The colour was due to methæmoglobin probably with some oxyhæmoglobin, and also in part to bile pigment. The evidence brought forward by Christophers and Bentley [1], and by Barratt and Yorke [2], indicates that the hæmoglobinuria in blackwater fever is the result of the presence of free hæmoglobin in the blood plasma (hæmoglobinæmia). Barratt and Yorke succeeded in producing hæmoglobinæmia and hæmoglobinuria in rabbits by injecting hæmolytic serum. It has been pointed out by Christophers and Bentley that the presence of hæmoglobin

in the serum is no proof of the existence of hæmoglobinæmia, since some lysis of the red corpuscles may occur during clotting.

The Treatment.—Every case received one or more doses of quinine daily during the hæmoglobinuria. This was given in the form of the bihydrochloride usually in doses of ten to fifteen grains, and, as a rule, intramuscularly, but when intravenous saline injections were given the dose was added to the saline in most cases. Some cases received fifteen grains daily for a week or longer, others were given doses of ten to fifteen grains at irregular intervals. When the tendency to vomiting had ceased, quinine hydrochlor. or quinine sulphate was given by the mouth. In two or three cases the temperature chart suggested that the quinine had some effect in reducing the fever. There was no evidence that the quinine increased or caused a relapse of the hæmoglobinuria. Saline injections were given regularly every four or six hours by the rectum in almost all cases. In the more severe cases, especially when the enemata were not retained, one or more intravenous saline injections of one to three pints was given with decided benefit. Other treatment consisted of a single dose of calomel (two grains) to several of the patients. Subcutaneous injections of digitalin, pituitrin, strychnine, and in Case 4 ten cubic centimetres of normal horse serum were given subcutaneously the day before the hæmoglobinuria ceased.

Post-mortem Examinations.—Post-mortem examinations of our three fatal cases all showed the presence of malarial pigment in the spleen and liver, and also dark blackish colouration of the apices of the pyramids of the kidneys. Microscopic sections showed blocking of the collecting tubules with hæmoglobin globules and renal cells full of large granules, and in addition dilatation of the convoluted tubules and capsules of Bowman. These changes were most marked in Cases 11 and 15, in which death was due to suppression of urine, and were present to a less degree in Case 12, in which the fatal issue appeared to be in part due to the extreme anæmia in addition to partial suppression of urine. The changes found in the kidneys agreed with those present in Barratt and Yorke's fatal cases.

The liver of Case 15, in which there was a high degree of jaundice affecting all the tissues, showed obstruction of the bile capillaries by inspissated bile.

In all three cases the contents of the gall-bladder presented a remarkable appearance. The gall-bladder was full of dark green, almost black bile of the consistency of very thick porridge. It was almost solid and after incision of the sac was turned out in a solid mass.

The Causation of Blackwater Fever.

Three chief hypotheses have been suggested to account for attacks of blackwater fever.

(1) That the attacks are due to a distinct specific infection which is acquired in malarial countries, probably because the infective agent is a protozoon inoculated into man by mosquitoes similar to those which intro-

duce the malarial parasites. There appears to be no direct evidence in favour of this suggestion. The hypothesis is rendered less probable by the occurrence of cases of blackwater fever in Great Britain and elsewhere, weeks or months after the subjects have left a malarial country, necessitating, according to this view, a long and variable incubation period. The fifteen cases of first attacks here recorded occurred one to five months after the patients had arrived in a non-malarial country.

(2) That quinine is the cause. Most advocates of this view postulate a previous malarial infection of considerable duration. The above cases do not throw much fresh light on this question. One patient, No. 15, stated that though he was supposed to have taken quinine regularly, he had "dodged it" and had taken no quinine for a week before the onset of blackwater fever. In most of the cases quinine had been taken for a long period before the attack, in some apparently with fair regularity. In others, an increase of the dose of quinine, or a renewal of its administration, had taken place one to seven days before the onset of hæmoglobinuria. In one case the patient took no quinine for seven days, and then took 30 grains, 60 grains, and 60 grains in three successive days; the attack of blackwater fever began on the evening of the second day on which sixty grains had been taken.

When the administration of quinine had been renewed or the dose increased shortly before the attack of blackwater fever this step was always taken as a result of a return of fever suggesting a relapse of malaria and, consequently, the two events have an equal claim to be regarded as the exciting cause of the attack.

Every case received at least ten grains of the bihydrochloride intramuscularly during the attack and most of them had several such injections of ten to twenty grains. In four cases, Nos. 3, 11, 12 and 15, intravenous doses of the bihydrochloride of quinine were given with one or more pints of saline solution. These intramuscular and intravenous injections did not appear to increase the hæmoglobinuria or do harm. It so happened that two of the three cases which died, Nos. 11 and 15, received most quinine intravenously, but these doses were given after the onset of suppression of urine, and with the saline solution which was being injected on account of the anuria. Case 3 received two doses intravenously; the hæmoglobinuria ceased on the same day that the second dose was given, the first dose having been given the day before. Other cases which ran a short and comparatively mild course received fifteen grains intramuscularly daily for as long as seven to ten days.

These facts relating to quinine administration, though not conclusive nor different in kind from previously recorded evidence on the subject, do not lend much support to the view that quinine evokes attacks of blackwater fever.

(3) That blackwater fever is due to repeated attacks of malaria.

In considering the association of malaria and blackwater fever there are three striking points which come into prominence:—

(i) The patient has suffered from attacks of malaria on and off for months or years before the onset of blackwater fever.

(ii) Recent attacks of malaria have usually occurred and the blackwater fever is often immediately preceded by a malarial attack, e.g., Case 10 (see Table I), during which parasites may be found in the blood. Moreover, the symptoms immediately ushering in the blackwater fever are, as a rule, indistinguishable from a malarial attack. The evidence does not, however, point to blackwater fever being one of the forms of "pernicious malaria," such as cerebral malaria, pernicious hepatic attacks with severe jaundice, or pernicious renal attacks with hæmaturia. In these latter cases parasites are usually very numerous in the peripheral blood. It seems rather that repeated attacks of malaria produce a condition in the body which predisposes to the lysis of the red corpuscles and resulting hæmoglobinuria as suggested by Christophers and Bentley (see also Stephens and Christophers) [5].

(iii) The parasites are absent or rapidly disappear from the peripheral blood during an attack of blackwater fever.

Our cases agreed with the previous accounts quoted above in these three points, but the absence of malarial parasites or their disappearance during the attack of blackwater fever in our cases was not remarkable since quinine was given in every case, often in large and repeated doses.

The close association of malaria with the blackwater fever attack in some of our cases is described above and all our cases had had frequent relapses of malaria.

Method of Estimating the Degree of Hæmoglobinuria.

Direct estimation of the amount of blood pigment in the urine by comparison with a colour standard is very unsatisfactory because the kind of pigment is not always the same and there is often a mixture of pigments. Moreover, some of the blood pigment is not in solution, but is found in the deposit. A modification of Sahl's method in which the hæmoglobin is first converted into acid hæmatin appeared to be a solution of the difficulty.

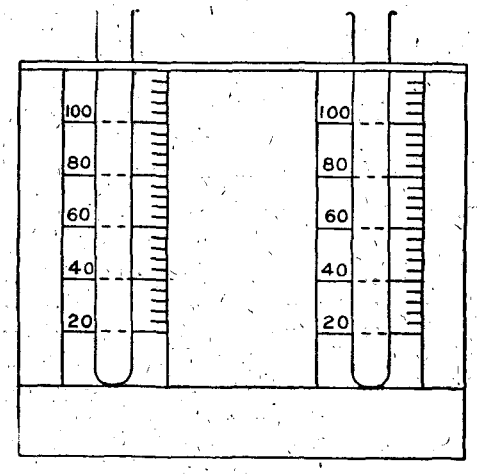
Method for Urine.—Since blackwater fever urine probably never contains more than twenty-five per cent of the amount of the hæmoglobin in normal blood (the highest figure which we obtained was 12·5 per cent) tubes about 1·5 cubic millimetres in diameter, i.e., twice that of ordinary hæmoglobinometer tubes, were used, and a standard solution of acid hæmatin was made with 0·5 per cent instead of one per cent of normal blood.

Several tubes of equal diameter were selected and calibrated with mercury at 1, 2, 5 and 10 cubic centimetres. The colour standard was prepared by taking 0·05 cubic centimetre of normal blood and making it up to 10 cubic centimetres with $\frac{N}{10}$ HCl saturated with chloroform as a preservative.

A wooden stand carrying a graduated scale as shown in the diagram was made and the scale so arranged that the 100 mark corresponded to the

10 cubic centimetre line on the tube, the 10 mark with the 1 cubic centimetre line, etc.

To make an estimation, 0·5 cubic centimetre of urine was placed in one of the tubes and 0·2 cubic centimetre of $\frac{N}{1}$ HCl added. The contents were well mixed and allowed to stand for a minute in order that the solution might become clear. Dilution was then carried out with water till the colour matched the standard. To obtain the percentage of hæmoglobin in terms of blood the number read off the scale must be divided by ten, since the amount of urine used is ten times that of the blood used in making the standard. Thus a reading of fifty means that the urine contains blood pigment equal to that in a five per cent solution of blood. (This we have called five per cent hæmoglobin.) If the percentage of hæmoglobin is small, less than two per cent, 1 cubic centimetre of urine should be taken for the test instead of 0·5 cubic centimetre and the reading divided by twenty.



That the whole of the hæmoglobin and altered blood pigment in the urine is really converted into acid hæmatin and correctly estimated by the above method was shown by the following experiments:—

(1) A solution of defibrinated rabbit's blood in water was taken and five cubic centimetres placed in two tubes A and B; to A was added 0·2 c.c. of water and to B 0·2 c.c. of one per cent solution of sodium nitrite. In tube B, methæmoglobin was found giving characteristic absorption bands.

The amount of blood pigment was then estimated in each by the method given above.

A. Exp. (1) 0·5 of A	+	0·5 c.c. $\frac{N}{10}$ HCl (mixed)	+	H_2O as diluent.					Estimated Hb	50 to 54 %
Exp. (2)	,,	+	,,	,,	+	,,	,,	,,	,,	48 to 52 ,,
B. Exp. (1) 0·5 of B	+	,,	,,	+	,,	,,	,,	,,		48 to 52 ,,
Exp. (2)	,,	+	,,	,,	+	,,	,,	,,	,,	48 to 52 ,,

The estimations before and after the formation of methæmoglobin were practically identical.

(2) The method was also tested by adding blood to acid and alkaline urines and estimating the hæmoglobin both before and after (1) placing the mixtures in the incubator at 37° C. for the night and (2) leaving it at the temperature of the laboratory (15° to 20° C.) for a fortnight; a few drops of chloroform were added to prevent the growth of bacteria. The estimated percentage in the different samples of urine did not alter during these experiments. These tests were necessary because when blood is mixed with urine and allowed to stand such changes take place in the colour and appearance of the urine that estimation of the hæmoglobin content by direct comparison with a colour standard is rendered impracticable, and it seemed possible that part of the hæmoglobin would have passed beyond the stage at which it could be estimated as acid hæmatin. Cf. observations by Barratt and Yorke [2] on changes in blood when mixed with urine.

This method of measuring the blood pigment in the urine was not adopted till most of the cases in the present series had recovered, but the results obtained in Cases 14, 15 and 16 are shown below. As a further illustration of the uses of the method the percentages found in a case of acute nephritis with deep red urine due to hæmaturia are also given. Case 14 (blackwater fever) after admission to hospital passed at least 1,640 cubic centimetres of dark porter-coloured urine between 3 p.m. April 4, and 6 a.m., April 6. The different specimens passed gave the following percentages of hæmoglobin (all the urine was not collected):—

April 4	3	p.m.	..	3·9 %	April 5	11.45	a.m.	..	3·9 %
	6.15	,,	..	3·8 ,,	,, 6	1	,,	..	2·7 ,,
	8.45	,,	..	4·3 ,,		6	,,	..	2·2 ,,

Case 15 suffered from almost complete suppression of urine; the estimated hæmoglobin in the few ounces excreted on April 5 and 6 respectively was 12·5 per cent and 5·6 per cent. These estimations may be rather excessive owing to the presence of bile.

On the other hand, Case 16, a very mild case of blackwater fever, excreted urine containing only 1·7 per cent and 0·7 per cent when the hæmoglobinuria was at its height. It must be remembered that a large part of the hæmoglobin excreted is found in the deposit, which must be well mixed with the urine before a sample is taken for the estimation.

Taking Case 14 as an example and assuming that as much as fifty ounces of urine (48 to 52 oz. measured) were passed daily during the five and a half days that the attack lasted and that the content throughout was 3·9 per cent of hæmoglobin, then the daily loss of blood by the kidneys would be 1·95 ounce or 11 ounces during the whole attack if the hæmoglobin of the blood were normal. The hæmoglobin in the blood during the last two days fell from fifty-four per cent on April 4 to twenty-four per cent on April 6, with a red-corpuscle count on the last day of 1,376,000.

A loss of this order does not seem nearly enough to account for the

extreme anæmia produced during blackwater fever attacks. It must be remembered, moreover, that in Case 14 the attack lasted an unusually long time.

Errors Due to Associated Abnormal Pigments.—A slight degree of error in the estimation may arise if the urine contains much additional abnormal pigment.

(1) Urobilin is frequently present in large amount in the urine of blackwater fever patients but not as a rule till after the hæmoglobinuria has ceased. In order to see whether a large error would arise from this cause a solution of hæmoglobin (blood diluted with water) was added to a very highly coloured urine containing much urobilin. It was found that the estimation was not appreciably affected if the hæmoglobin content was at least two per cent, since in this case the urine was diluted not less than four times during the estimation.

(2) Urochrome may in the same way cause rather too high a reading, but in the case of a dark yellow urine which was tested, it did not alter the estimate if at least two per cent hæmoglobin was present.

(3) Bile pigment if present in large amount may affect the estimation. If the amount of bile pigment is very large, especially if the hæmoglobin content is low, the method is inapplicable. If a large amount of bile-pigment is present and the percentage of hæmoglobin is two per cent or more, the difficulty may be obviated by taking 0·25 cubic centimetre of urine instead of 0·5 cubic centimetre. We have, however, only once been able to detect bile in the urine of blackwater fever, and apparently it has only rarely been found judging from the records to which we have access.

Our observations have led us to certain definite conclusions regarding the causation, pathology, and treatment of blackwater fever. We feel some diffidence in expressing our views, because our experience has been restricted to one set of conditions, and we have had only limited access to the literature of the subject. Our observations in the main support the views of Stephens, Christophers and Bentley, Barratt and Yorke, and others.

Conclusions.

(1) Blackwater fever is due to malaria.

(2) It is predisposed to by a long standing malarial infection with repeated relapses.

(3) An attack of blackwater fever is precipitated by a relapse or recurrence of malaria.

(4) The ascertained *maximum* and *minimum* intervals in different cases in our series between the arrival in a malarial country and the first attack of blackwater fever were *maximum* ten years, *minimum* seven months; between the first recognized attack of malaria and the first attack of blackwater fever were *maximum* ten years, *minimum* fifty days; between the arrival in a country which was non-malarial and free from *anopheles* and

the first attack of blackwater fever were *maximum* five months, *minimum* one month.

(5) Quinine in the class of cases with which we have met has no share in producing blackwater fever, nor has quinine treatment during or after the attack any effect in prolonging or reproducing hæmoglobinuria.

(6) The jaundice of blackwater fever is certainly in some cases due to bile-pigment in the circulation.

(7) It is possible to estimate the total amount of blood-pigment in the urine, whether in solution or in the deposit, after converting it into acid hæmatin (modified Sahli's method). The amount of hæmoglobin lost by the kidneys is much greater than would be ascertained by an attempt at direct hæmoglobin estimation of the urine. We are convinced, however, that the kidneys excrete only part, possibly a small part, of the hæmoglobin which is lost in an attack of blackwater fever.

(8) The treatment which appeared to be of most value was intravenous or rectal administration of physiological salt solution (NaCl 0·9 per cent). Whether quinine is of value or not was difficult to decide, since all our cases had some quinine and the intensity of the attack was no doubt different in each case.

Our best thanks are due to the medical officers of St. Andrew's and St. David's Hospitals, who were in charge of the cases of blackwater fever, for permission to publish their clinical notes, and for their courtesy and constant assistance. Especial thanks are due to Major Campbell, R.A.M.C., Captain Fannin, R.A.M.C., and Captain Allen, R.A.M.C., and Dr. Bell, Dr. Gilchrist, Dr. Gorrie, and Dr. Rougvie, medical officers attached Royal Army Medical Corps, of St. Andrew's Hospital, and to Lieutenant Barrett, R.A.M.C., and Dr. Hollway, medical officer attached Royal Army Medical Corps, of St. David's Hospital.

Dr. Hollway not only supplied careful clinical notes of six cases, but by examining many blood films was able to establish a diagnosis of the species of malarial parasite in all her cases.

We were very fortunate in having the advice of Colonel A. E. Garrod, A.M.S., in connexion with the spectroscopic work and the identification of urinary pigments.

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