

THE EXCRETION OF UROBILINOGEN IN THE STOOLS AND URINE DURING MALARIAL INFECTION

By A. DE VRIES, M.D.

With the technical assistance of F. Schiffer, Dipl. Ing.

A VARIABLE degree of blood destruction over and above that occurring physiologically is a constant phenomenon in attacks of malaria. Although blood destruction is always increased in a malarial attack, clinical signs of hemolysis (jaundice, anemia, etc.) are not always present.

The clinical syndrome of hemolytic anemia in malaria is known in its severest form as blackwater fever, which is characterized by the acute development of a marked anemia, hemoglobinuria, intense jaundice, and marked reticulocytosis. In less severe hemolysis, there is only slight jaundice and moderate anemia, accompanied by a slight rise in the number of reticulocytes and by an increased excretion of urobilinogen in the urine. However, in these cases the hemolytic nature of the jaundice is not generally accepted. Stitt^{2b} remarks: "It is not improbable, although this point is often disputed, that the yellow tinge of the skin and the sclerae often observed in malaria is due to the tinting of the tissues by the liberated hemoglobin and not as popularly believed to biliousness or cholaemia from bile absorption."

Hemolysis in its slightest form may at times be manifested, according to Stitt and Manson Bahr, by a positive indirect van den Bergh reaction in the blood, even without clinical jaundice. In addition, there are cases of malaria in which neither clinical signs nor laboratory findings pointing to hemolysis are observed. In this mild form, if treated early, no appreciable anemia develops, no jaundice is observed, no increase of bile pigments in the blood is found, even urobilinogenuria may be absent and reticulocytosis may not be encountered.

The purpose of this paper is to show that increased blood destruction in malaria is manifested by an increased excretion of urobilinogen in the stools, even when all the above recorded signs of hemolysis are lacking. These studies give further indication of the importance of the fecal urobilinogen output as a criterion of the degree of blood destruction.

MATERIAL AND METHODS

The material upon which this study was made comprises 10 consecutive cases of malaria. One patient suffering from benign tertian malaria was studied on two admissions, the second time during a relapse appearing 5 weeks after the first admission. The age of the patients varied from 19 to 67 years. Two were women, 7 men. None of the patients suffered from any other disease prior to admission, and no complicating conditions were present during hospitalization. In 6 cases the diagnosis was benign tertian malaria, in 4 malignant tertian. All the cases of

From the Medical Department, Division B, Rothschild Hadassah University Hospital, and from the Laboratory of Pathological Physiology, Hebrew University, Jerusalem, Palestine. The work was carried out with the help of the Jacob Kerbel Scholarship Grant.

benign tertian were relapses, and 2 of the patients with malignant tertian had their first attacks. In all of them the diagnosis was established by the finding of parasites in the blood.

The patients were admitted on the first to the sixteenth day after the beginning of the illness. All of them, except one, had fever either on admission or on the previous day. Only one patient (no. 3) was admitted 3 days after defervescence; nevertheless, significant observations on urobilinogen excretion could be made during hospitalization.

The number of paroxysms in the cases of benign tertian malaria varied from 1 to 3. Two patients with malignant tertian malaria had 2 and 3 paroxysms respectively, and 2 others had continuous fever. The total period in which fever occurred varied from 3 to 6 days in the patients with benign tertian malaria, and from 6 to 19 days in those with malignant tertian. Medication consisted of quinine and atabrin. Quinine as the only drug was given in 2 cases; atabrin as the only drug in 2 other cases. In 5 cases medication consisted of a course of quinine followed by atabrin. The maximal dose of quinine given was 11.4 grams in 9 days, the maximal dose of atabrin 2.8 grams in 7 days. No medication was given in case no. 3, as no parasites were found. In all cases asexual forms disappeared rapidly from the blood with medication. In only 2 cases (nos. 7 and 9) were gametocytes present after treatment.

Evidence of increased blood destruction was obtained by:

1. Repeated determinations of hemoglobin and red cell values.
2. Repeated determinations of the reticulocyte count.
3. Repeated examination of the icterus index and the qualitative van den Bergh reaction in the serum.
4. Quantitative determination of the amount of urobilinogen excreted in the stools and in the urine during and after the attack.

The red cell and hemoglobin values were estimated in venous blood. The red cells were counted in a Spencer counting chamber; two determinations with two pipets were performed and the average taken. Duplicate values differing by more than 200,000 per mm^3 were discarded. The hemoglobin content of the whole blood was determined with the Stufenphotometer according to the method of Heilmeyer³ and expressed in grams per 100 cc.

The reticulocytes were examined by the Nile blue method. A smear of a 1 per cent aqueous solution of Nile blue was made on a slide and allowed to dry. A cover glass, on which was placed a tiny drop of blood, was gently pressed on the Nile blue smear and the count performed after 20 to 30 minutes. At least 500 red cells were counted. The reticulocytes were calculated as the percentage of the red cell count, as well as the total number per mm^3 . (The normal value: less than 1 per cent.)

The icterus index was measured by the modified Meulengracht method (the highest normal is 7).

The quantity of urobilinogen excreted in the stools and in the urine was determined by the method described by Watson.⁴ After discarding the first stool produced after admission, the stools were collected during 48 hour periods and were

examined for at least two periods (in only a few instances was one collection period continued for 72 hours). The samples were kept in a dark place in a container, surrounded by ice. Determinations were performed at the end of each 48 hour period. The urine was collected during 48 hour periods under the same conditions. The values were expressed as milligrams of urobilinogen excreted in 24 hours. The normal values found by the author ranged from 50 to 180 mg. for the stools and up to 2 mg. for urine per 24 hours (5 normals). The normal values given in the literature range from 100 to 280 mg. per 24 hours (Dameshek et al.⁵: 66 to 180 mg. per 24 hours; the highest value given by Watson⁶ is 280 mg. per 24 hours).

RESULTS

THE TOTAL EXCRETION OF UROBILINOGEN PER DAY

In all of the 10 cases examined, the total excretion of urobilinogen (in stools plus urine) was found to be increased. The highest total daily excretions for each patient calculated from a 2 day period varied from 370 to 1142 mg. (431, 1142, 510, 370, 851, 438, 949, 367, 518, 477). The highest total daily excretions calculated from 4 day periods varied from 325 to 822 mg. (375, 822, 412, 314, 586, 361, 734, 325, 475). Thus in 9 cases the excretion was found to be increased during a continuous period of at least 4 days, during which two 48 hour samples were examined. This excludes accidental fluctuations. (In case 9 the total excretion could be measured only during a 2 day period at the beginning of the hospitalization.)

Factors which might possibly influence the excretion of urobilinogen are diarrhea and constipation, diet and fever. None of our patients had diarrhea or severe constipation, and none received laxatives. Since ingestion of a large quantity of fat may increase hemolysis⁷ all of our patients were given a normal diet with an average fat content of 60 grams a day. In the writer's experience fever itself does not increase the quantity of urobilinogen excreted in the stools (normal values were obtained in Malta fever and typhoid fever). This corresponds to the findings of Vaughan and Saifi,⁸ who generally did not find an increased excretion of urobilinogen in infectious diseases. Medication cannot be held responsible for the increased excretion, since during the course of the treatment the excretion of urobilinogen gradually diminishes. In case no. 3 no medication was given at all and the urobilinogen excretion in the stools was found to be increased.

It is thus evident that the increase in the total excretion of urobilinogen observed in our cases can only be the sequel of an increased red cell destruction due to the malarial infection.

The earliest period in which the total daily excretion of urobilinogen could be measured was the third and the fourth days of the illness (case 1). Although this patient came in within 24 hours after the beginning of the attack, the necessity of discarding the first stools made the determination possible only on the third and the fourth days, when it was found to be increased (319 mg. per 24 hours). An increased total daily excretion of urobilinogen was observed 2 to 7 days after defervescence. In 6 cases a return to practically normal values was observed on the seventh to twenty-first day (nos. 1, 2, 4, 6, 9, 10). In two cases (5, 8) the last determinations on the eleventh and ninth days still showed an increased value,

No.	Initials	Age	Sex	Malaria	No. of paroxysms	Highest fever degree Celsius	Parasites present on day of illness	Therapy			First count			Lowest values observed			Fall in			Highest reticulocyte count			Jaundice					Urobilinogen excretion per 24 hours in milligrams			
								Drug	Dose in grams	On days	Red cells in millions/mm ³	Hemoglobin in gram %	On day	Red cells in millions/mm ³	Hemoglobin in gram %	In days	%	Total per mm ³	On day	Day of illness	Clinical jaundice	Icterus index	Direct	Van Den Bergh reaction	Indirect	On days	In stools	In urine	Total		
1	D. F.	67	F.	Vivax	1	4	39.5	+1 -5	1.8	1	6	3	4.43	11	6	4.10	11	0.33	3	6	246,000	6	2	50	+	+	3-4	271.48	319		
2	J. M.	32	M.	"	3	5	40.2	+5 -8 -10	6	5	8	5	5.35	12.4	11	4.94	11	0.41	6	1.3	65,500	14	6	15	traces	traces	6-7	495	7	502	
3	Sh. K.	21	M.	"	2	3	40	+1	7	4	69	13.2	7	4.69	13.2					<1	<47,000	5-12	6	5	-	-	6-8	305	10	315	
4	M. K.	19	M.	"	2	4	40	+3 +4 -8	5.7	4	7	4	5.02	13.7	11	4.80	13	0.22	7	<1	<50,000	4-11	4	20	traces	traces	4-5	58	370		
5	M. L.	28	M.	"	3	5	39.2	+5 -8 -10	3.9	5	7	6	4.44	12.9	10	4.31	13.2				<1	<44,000	6-10	5	-	traces	traces	6-7	825	26	851
6	M. L.	28	M.	"	3	5	40	+3 +6 -12	10.8	3	9	4	5.16	14.8	9	4.48	13	0.68	5	<1	<52,000	3-10	6	10	-	-	4-6	420	17.5	438	
7	K. P.	27	M.	Quartan	3	5	41	+4 +5 -10 Gam. 12	11.4	4	12	4	6.10	16	13	5.00	14.3	1.10	9	<4	<30,000	4-13	4	5	-	-	5-6	50.3	949		
8	Ts. A.	30	M.	"	2	4	39.4	+4 -8	1.5	4	6	6	5.25	13.9	8	5.13	13.4				1.6	82,000	8	7	5	-	-	5-7	22	367	
9	M. M. N.	18	F.	"	19	40	+16 Gam. 24	2.1	17	24	16	3.15	8.4	16	3.15	8.4				8	256,000	17	19	6	-	traces	traces	17-18	516	1.8	518
10	M. L.	21	M.	"	8	40.2	+5 -6 -9	6.7	5	9	8	4.75	12	11	4.10	10.2	0.65	0.8	3	5.4	231,000	15	8	15	+	+	6-8	426	46	472	

but there was a definite tendency to become normal. These findings furnish additional proof that the increased excretion of urobilinogen found in malaria is due to the infection itself. In cases 3 and 7 the last values obtained were still high, but it may be assumed that a return to the normal would have been found had the examination been extended for a longer period.

The amount of urobilinogen excreted in the stools forms, of course, the major part of the total excretion. The highest daily excretion in the stools, calculated as the average of a 2 day period excretion, was 1136 mg.

THE EXCRETION OF UROBILINOGEN IN THE URINE

In all cases except one (9) the daily excretion of urobilinogen in the urine was found to be significantly increased. The highest daily excretion observed in a 2 day period was 58 mg. The amount of urobilinogen excreted in the urine returned to practically normal values in 3 cases (1, 7, 8) after about 8 days. In 3 cases (4, 5, 10) the excretion decreased markedly but did not reach the norm during 10 to 13 days. In cases 2, 3, and 6 there was also a tendency to decrease but to a lesser degree. The decline in the excretion of urobilinogen in the urine preceded that in the stools. There was no correlation between the excretion of urobilinogen in the stools and in the urine. For example, on comparing the excretions in cases 1 and 9, the quantities of urobilinogen excreted daily in the stools were 423 (case 1) and 516 mg. (case 9), and in the urine 48 mg. (case 1) and 4.5 mg. (case 9) respectively.

ANEMIA

Although increased blood destruction was present in all cases examined, significant changes in the red count were observed in only 6 cases (1, 2, 5, 6, 9, 10). An appreciable fall in the red cell and hemoglobin values during the period of study was observed in only 3 cases (6, 7, 10). There was no correlation between the total quantity of urobilinogen excreted and the fall in the red cell and hemoglobin values. Sufficient explanation for this is seen in the fact that the level of the red blood cell values before the beginning of the illness could not be determined, that changes in water balance may mask actual changes in the blood count, and finally that an increased blood destruction may be compensated by increased regeneration.

No correlation was found between the degree of anemia and the amount of urobilinogen excreted in the urine (compare cases 9 and 4).

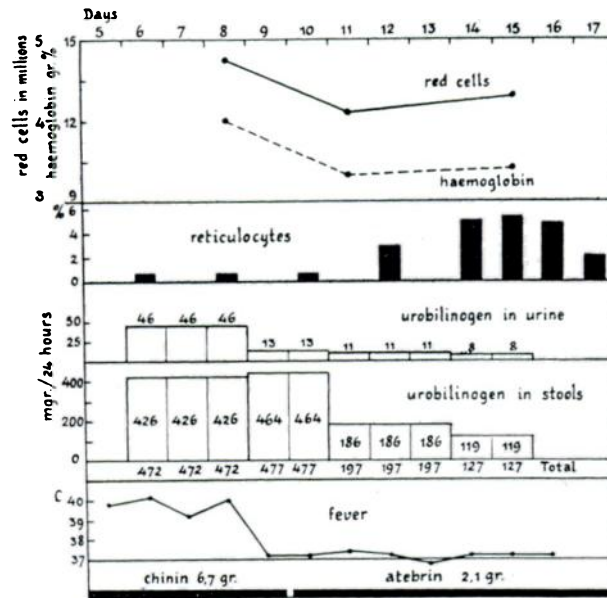
JAUNDICE AND BILE PIGMENTS IN THE BLOOD

In only 1 case (no. 1) was definite jaundice noted clinically, corresponding with a high icterus index (50) and a positive indirect van den Bergh reaction. In the other 9 cases clinical jaundice was not observed, and in none of them was the icterus index higher than 20; the indirect van den Bergh reaction was positive in only 1 of these cases. In 4 cases only traces of indirect reacting bilirubin were found in the blood. In the remaining 4 cases no laboratory findings suggestive of latent jaundice were revealed. It is thus evident that there is no correlation between the total amount of urobilinogen excreted and the degree of jaundice, the height of the icterus index or the presence of an indirect van den Bergh reaction. Nor was there a

relationship between the amount of urobilinogen excreted in the urine and the laboratory evidence of jaundice.

RETICULOCYTES

An increased number of reticulocytes was found in only 3 cases (1, 9, 10) with the lowest red cell values. The increased excretion of urobilinogen was not associated with reticulocytosis (cases 7, 2) in the absence of anemia. It seems that in some cases the appearance of reticulocytosis may be delayed by the malarial in-



fection. In case 10, for example (see graph), the reticulocytes began to rise 3 days after defervescence and after the disappearance of the parasites from the blood. This corresponds to the observations of Castle and Minot⁹ and of Blackie.¹⁰ However, reticulocytes may also appear while fever is still present and parasites are found in the blood (case 9).

DISCUSSION

In contrast to the numerous reports on the excretion of urobilinogen in the urine in malaria, studies on the total excretion (in stools and urine) are scarce.

Manson Bahr¹¹ states: "The corresponding pigment in the faeces (hydrobilirubin) is increased to twenty times the normal amount, as long as there is fever and parasites are present in the blood."

Eppinger¹² mentions a case of relapsing malignant tertian malaria in which the daily amount of urobilinogen excreted in the stools was 330 and 364 mg. (the upper normal range according to this author is 140 mg. daily).

An increased daily total excretion of urobilinogen was found in all our cases. This excess of excretion was present even when no appreciable anemia or other sign of hemolysis was observed. Although the amount of excreted urobilinogen

cannot be used as an exact indication of the quantity of red blood cells destroyed, the observed lack of correlation between jaundice and total urobilinogen excretion leads to the assumption that the amount of red blood cells destroyed is not the only factor responsible for "hemolytic jaundice" in malaria. As pointed out by Miller, Singer, and Dameshek,⁵ none of the various indices of increased blood destruction is specific, except for the fecal urobilinogen output, which gives unequivocal evidence of an increased breakdown of blood.

The additional factor responsible for the development of jaundice in hemolytic conditions is probably the inability of the liver to deal with the bilirubin formed (Watson¹³). The liver in malaria was studied systematically by some investigators and a disturbance in liver function was revealed in most cases (Kopp et al.,¹⁴ Greene et al.,¹⁰ Mirsky et al.¹⁶).

No attempt was made in our study to correlate jaundice and routine liver function tests as they bore no constant relationship to the state of the liver. Some indication of the functional capacity of the liver may be furnished by the amount of urobilinogen excreted in the urine (Watson¹⁷).

Numerous authors are of the opinion that the amount of urobilin or urobilinogen in the urine is always increased in malaria (Atkinson,¹⁸ Antic and Neumann,¹⁹ Saupé,²⁰ Reynolds,²¹ Gordon,²² Ballerstedt²³). Other investigators claim that the amount of urobilinogen in the urine is increased in some cases of malaria and not in others (Uvedale Owen,²⁴ Plehn,²⁵ Stitt²). Uvedale Owen²⁴ states that urobilinogenuria is generally more excessive in malignant tertian malaria and that the quantity of urobilinogen excreted in the urine bears no relationship to pyrexia, enlargement of the spleen, or the number of parasites present in the blood. His studies indicate that quinine increases the excretion of urobilinogen in the urine and that this increase may appear 8 to 37.5 hours after the first dose.

According to Plehn²⁵: "Urobilinuria in malaria is only a symptom of liver-disturbance. . . . Hence urobilinuria need not necessarily be found in every case of malarial fever, for malaria may exceptionally spare the liver." The results of our quantitative determinations show that the excretion of urobilinogen in the urine in malaria may or may not be increased. In 7 of 10 cases it was found to be markedly increased during the period of fever and decreased rapidly after defervescence. In 1 case (9) it was found to be practically normal, although fever was present and parasites were found in the blood. In 2 cases there was a slight increase of urobilinogen excretion in the urine (cases 2 and 3).

No correlation was seen between the amount of urobilinogen excreted in the urine and that excreted in the stools. It thus seems that red cell destruction alone cannot account for the increased excretion of urobilinogen in the urine and that a disturbance in the capacity of the liver to remove the absorbed urobilinogen from the intestine must be assumed. In this respect the bile pigments in malaria behave as in other hemolytic diseases (Watson¹⁷). Fever itself cannot be held responsible for the urobilinogenuria, as normal values were found in case 9 in spite of hyperpyrexia and the same observation was made in other febrile diseases such as Malta fever and typhoid fever (see also Watson⁶). No difference was found between

benign and malignant tertian malaria as to the quantity of urobilinogen excreted in the urine. On the other hand, the excretion of urobilinogen in the feces is constantly increased.

SUMMARY

In 10 cases of malaria (6 benign tertian, 4 malignant tertian), the excretion of urobilinogen in the stools and in the urine was studied. In all 10 cases the amount of urobilinogen excreted in the stools was found to be increased. After defervescence and disappearance of parasites from the blood the excretion gradually declined. The increased excretion of urobilinogen in the stools was *the constant and sometimes the only evidence of increased blood destruction* occurring at times in the complete absence of jaundice and reticulocytosis. Increased excretion of urobilinogen in the urine was not a constant feature.

It is suggested that the development of jaundice and of urobilinogenuria is due not only to the liberation of pigments by the hemolysis, but to a disturbance in the liver function.

This study lends further confirmation to the concept that the only unequivocal evidence of increased blood destruction is shown in an increased output of urobilinogen in the feces.

The author is indebted to Dr. M. Rachmilewitz for his suggestions and criticisms.

REFERENCES

- ¹ BOYD, M. F.: Present day problems of malaria infections. *J. A. M. A.* 124: 1179-87, 1944.
- ² STRONG, R. P.: Stitt's diagnosis, prevention and treatment of tropical diseases. Philadelphia: The Blakiston Co., ed. 6, 1, 19, 54, 1942.
- ³ ZEISS, C., AND KREBS, W.: Klinische Kolorimetrie mit dem Pulfrich-Photometer. Leipzig, 1935.
- ⁴ WATSON, C. J.: The average daily elimination of urobilinogen in health and in disease, with special reference to pernicious anemia. Standardization of method based on mesobilirubinogen (H. Fischer). *Arch. Int. Med.* 47: 698-26, 1931.
- ⁵ MILLER, E. B., SINGER, K., AND DAMESHEK, W.: Use of daily fecal output of urobilinogen and the hemolytic index in the measurement of hemolysis. *Arch. Int. Med.* 70: 722-37, 1942.
- ⁶ WATSON, C. J.: Studies of urobilinogen: Urobilinogen in the urine and feces of subjects without evidence of disease of the liver or biliary tract. *Arch. Int. Med.* 59: 196-205, 1937.
- ⁷ JOSEPHS, H. W., HOLT, L. E., JR., TIDWELL, H. C., AND KAJDI, C.: The influence of dietary fat upon the excretion of urobilinogen. *Bull. Johns Hopkins Hosp.* 71: 84-95, 1942.
- ⁸ VAUGHAN, J. M., AND SAIFI, M. F.: Haemoglobin metabolism in chronic infections. *J. Path. & Bact.* 49: 69-82, 1939.
- ⁹ MINOT, G. R., AND CASTLE, W. B.: Interpretation of reticulocyte reactions: Their value in determining potency of therapeutic materials, especially in pernicious anemia. *Lancet* 2: 319-30, 1935.
- ¹⁰ BLACKIE, W. K.: The reticulocytes in blackwater fever. *Tr. Roy. Soc. Trop. Med. & Hyg.* 28: 571-78, 1935.
- ¹¹ MANSON-BAHR, P. H., ED.: Manson's tropical diseases: A manual of the diseases of warm climates. Baltimore: William Wood & Company, ed. 11, 1940.
- ¹² EPPINGER, H., AND WALZEL, P.: Die Krankheiten der Leber mit Einschluss der hepatolienalen Affektionen. Leipzig: Georg Thieme, 1926.
- ¹³ WATSON, C. J.: Hemolytic jaundice and macrocytic hemolytic anemia: Certain observations in a series of 35 cases. *Ann. Int. Med.* 12: 1782-96, 1939.
- ¹⁴ KOPP, I., AND SOLOMON, H. C.: Liver function in therapeutic malaria. *Am. J. M. Sc.* 205: 90-97, 1943.

- ¹⁶ GREENE, C. H., AND BRUGER, M.: (a) The functional study of the liver and its clinical evaluation. *New York State J. Med.* 43: 318-22, 1943. (b) Cit. Greene, C. H.: *Arch. Int. Med.* 73: 349, 1944. (c) Review: Diseases of the liver and the biliary tract.
- ¹⁶ MIRSKY, J. A., VON BRECHT, R., AND WILLIAMS, L. D.: Hepatic dysfunction in malaria. *Science* 99: 20-21, 1944.
- ¹⁷ WATSON, C. J., in: Downey, Hal, ed., *Handbook of hematology* in 4 volumes. New York: Paul B. Hoeber, Inc., 1938, vol. 4.
- ¹⁸ ATKINSON, J. M.: A clinical test for malarial fever. *Lancet* 1: 1802-03, 1913. (Cit. Owen, 24.)
- ¹⁹ ANTIC, D., AND NEUMANN, S.: Über Malaria und die Diagnostische Bedeutung der Urobilinogenurie bei Malaria. *Centralbl. f. Bakt.* 67: 359, 1918. (Cit. Owen, 24.)
- ²⁰ SAUBE, E.: Über Urobilinogenurie bei Malaria. *Arch. f. Schiffs- u. Tropen-Hyg.* 22: 17-27, 1918. (Cit. Owen, 24.)
- ²¹ REYNOLDS, R. E.: Urobilinuria with continuous malarial fever. Abstract, *J. A. M. A.* 74: 1358, 1920.
- ²² GORDON, R. M.: The value of urinary examinations in the diagnosis of malaria. *Ann. Trop. Med.* 18: 351-62, 1924.
- ²³ BALLERSTEDT: Das Verhalten des Urobilins bei Malaria. *Arch. f. Schiffs- u. Tropen-Hyg.* 28: 100-119, 1924. (Cit. Owen, 24.)
- ²⁴ OWEN, D. UVEDALE: Observations on the output of urobilinogen in malaria and the influence of quinine upon it. *Ann. Trop. Med.* 22: 461-502, 1928.
- ⁵ PLEHN, A.: The diagnosis of latent malaria. *Brit. J.* 2: 1357-58, 1908.