MALARIAL PIGMENT (HEMATIN) AS AN ACTIVE FACTOR IN THE PRODUCTION OF THE BLOOD PICTURE OF MALARIA.*

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In a recent article[1] the author submitted evidence to show that hematin intoxication in the rabbit was characterized by a paroxysm analogous to that of malaria, and a study of the effect of this pigment upon the blood is offered as further evidence of the rôle that hematin plays in malaria. While particular attention has here been directed to the formed elements of the blood, other important effects have been noted, such as the effect upon the coagulation time and the bleeding time.

The hematin, the hematin solutions, the doses, and the technique used in these experiments were identical with those used in previous experiments. In fact, many of the observations upon which this report is based were made in the course of experiments described in my former article. The experiments have been graded so as to determine the effect of a single injection of various amounts of alkaline hematin, and finally to follow the changes produced by injections repeated at various intervals over a period of days or even weeks. All phases of the experiments were controlled in two ways. One series of controls was made by injecting rabbits with the same amount of the solvent for the hematin (1 or 2 per cent. bicarbonate of soda dissolved in 0.85 per cent. sodium chloride solution) per kilo of body weight, and a second series of controls was made by first injecting the alkaline salt solution, and, after the

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[1] Brown, W. H., *Jour. Exper. Med.*, 1912, xv, 579.

animal's blood had returned to normal, injecting the hematin solution. This procedure was followed with only a few animals.

The Effect of Hematin upon the Red Blood Corpuscles.—The red blood corpuscles, although decidedly less subject to the toxic influence of hematin than either the leucocytes or the platelets, may be markedly reduced in numbers by the intravenous injection of hematin. A single injection of less than fifteen milligrams of hematin per kilo of body weight seldom produces any demonstrable effect upon the red cell count. If there is a decrease, regeneration is sufficiently rapid to obscure the change and this is probably the case. An injection of as much as twenty milligrams of hematin per kilo, however, usually shows, within a few hours, a decrease of as much as 1,000,000 cells, and frequently more. This decrease is still only transient although a reduction may be evident for as long as twenty-four hours.

The daily repetition of a dose of even ten milligrams of hematin per kilo will ultimately produce a definite anemia, and the larger the dose the more marked is the anemia. In well marked anemia the cells are irregular in size, the color is variable, and immature cells are present. The most common of these is the young basophilic cell, although nucleated red cells may be present in considerable numbers. Such an instance is illustrated by the following experiment:

Rabbit 5.—Albino, female; weight, 2.85 kilos. Within seven days this animal received eleven injections averaging 10 mg. of hematin per kilo of body weight. The red cells showed the following changes:

May 2, 1911. Red blood corpuscles, 7,596,000; nucleated red cells, 3 (normoblasts).

May 9. Red blood corpuscles, 3,200,000; nucleated red cells, 179; normoblasts, 153; intermediates, 21; megaloblasts, 5.

This experiment is cited merely to show to what degree the destruction of red cells can be pushed under favorable circumstances. The usual reduction, however, does not exceed one third of the normal count and nucleated cells are comparatively few.

As regards the hemoglobin, with single injections and slight reduction in the red cells, the hemoglobin index (Sahli) is not altered from the normal. With more pronounced destruction of red cells, and especially with the influx of immature cells into the circulation, the hemoglobin index is usually reduced, but it may be normal or rarely increased above the normal. In some of these cases it is possible that hematin still in the circulation has influenced the hemoglobin determinations.

The anemia produced by the intravenous injection of alkaline hematin is due entirely to the hematin, as the alkaline salt solution has been found entirely incapable of producing the slightest degree of anemia, even when used in much larger amounts than were employed with the hematin. In fact, the only effect upon the red blood corpuscles, observed in the control animals, was in the opposite direction. Most of the animals showed no appreciable effect, while a few showed a very definite increase in the red cell count with a corresponding increase in the percentage of hemoglobin.

The mechanism of destruction of the red cells is by no means clear. *In vitro,* the alkaline hematin solution produces no hemolysis and this would be expected on account of the hypertonicity of the solution. In the animal body, on the contrary, some degree of hemolysis has been detected in many instances. The hemolysis, however, does not depend entirely upon the amount of hematin injected, as marked hemoglobinemia was noted in one case after an injection of ten milligrams of hematin per kilo of body weight, and was very slight or not demonstrable in several other instances where the dose of hematin was as much as twenty milligrams per kilo. The evidence at hand is not sufficient to warrant an expression of opinion as to the factors that determine the hemolytic action of hematin.

Microscopic study of the tissues shows that red cells are also destroyed intact as numbers of these cells are found included within phagocytic cells.

The Effect upon the Leucocytes.—The leucocytes, unlike the red cells, are markedly effected by simple alkaline salt solutions as well as by solutions of hematin, and this circumstance has added to the difficulties of establishing the leucocyte picture of hematin intoxication. The intravenous injection of alkaline salt solution produces a rapid decrease in the total number of leucocytes. Within three to five hours the count has again risen to normal, the rise continuing to a definite leucocytosis which lasts only a few hours and then slowly falls to normal. A count taken twenty-four hours after the injection practically always shows a normal number of leucocytes. Differential counts (500 cells) show that all types of cells participate in these changes to some degree. The polymorphonuclear amphophile, however, is the cell that is most influenced. These cells show an immediate absolute reduction and frequently a relative reduction as well; they then increase rapidly so that their actual number may be well above the normal, while all other cells are still decreasing or at their lowest point; then follows a gradual fall to normal. Next to the polymorphonuclear cells, the large mononuclear and transitional group shows the most pronounced effect, an effect that is characterized by a rapid decrease with a slow recovery which continues well above the normal, both as regards percentage and actual numbers, but rarely exceeds 8 to 10 per cent. The small mononuclears decrease but slowly and regenerate even more slowly. The eosinophiles and basophiles are inconstant in their behavior, the former being but little affected while the latter may show marked fluctuations, characterized by an initial decrease and a marked subsequent increase.

By repeating the injections of alkaline salt solution daily, for a week or more, this picture is altered but little. The most important change is to be found in an increase of the mononuclear cells at the expense of the polymorphonuclears. The large mononuclears under these conditions may reach 12 to 14 per cent.

When the effect upon the leucocytes of a single dose of ten to fifteen milligrams of hematin per kilo is compared with the effect of an equivalent volume of alkaline salt solution, the differences that can be detected are but slight. To facilitate comparison the following abbreviated protocol and series of counts from one of my experiments are given.

Rabbit 48.—Belgian, male; weight, 2 kilos.

November 13, 1912. 9.30 A. M. White blood corpuscles, 6,600. Injected 6.6 c.c. of 2 per cent. sodium bicarbonate in 0.85 per cent. sodium chloride solution. The leucocytes were counted at intervals of one, three, five, and twenty-six hours.

November 20. 9.30 A. M. White blood corpuscles, 8,800. Injected 6.6 c.c. of hematin solution (10 mg. of hematin per kilo). The leucocytes were counted at intervals of one, three, five, and twenty-six hours. Complete counts are given in table I.

TABLE I.

The Leucocyte Reactions Produced by a Single Dose of Ten Milligrams of Hematin per Kilo of Body Weight and by a Corresponding Volume of Alkaline Salt Solution.

Time.	Leucocyte count.	Polymorphonuclear cells.		Small mononuclear cells.		Large mononuclear cells.		Eosinophiles.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Nov. 13, 1912, 9.30 A.M.	6,600	54.0	3,564	32.8	2,164	6.4	422	0.8	52	6.0	396
10.30 A.M.	3,600	56.0	2,016	38.4	1,382	1.6	57	0.4	14	3.6	129
12.30 P.M.	5,800	82.0	4,756	15.6	904	2.0	116	0.0	0	0.4	23
2.30 P.M.	8,600	78.8	6,776	15.2	1,307	4.4	378	0.0	0	1.6	137
Nov. 14, 11.30 A.M.	6,600	57.2	3,775	17.4	1,148	9.4	620	0.4	26	15.6	1,029
Nov. 20, 9.30 A.M.	8,800	45.6	4,012	39.2	3,449	7.2	633	1.6	140	6.4	563
10.30 A.M.	5,800	25.5	1,479	71.0	4,118	2.5	145	1.0	58	0.0	0
12.30 P.M.	13,000	75.2	9,776	21.2	2,756	2.4	312	0.0	0	1.2	156
2.30 P.M.	9,200	77.6	7,139	17.2	1,582	3.6	331	0.4	36	1.2	110
Nov. 21, 11.30 A.M.	6,800	26.4	1,795	53.2	3,617	15.2	1,033	0.0	0	5.2	353

While many differences in the two series of counts in table I are apparent, the only feature to which I attach any importance is the relatively larger number of mononuclear cells, including large and small mononuclears, in the hematin series as compared with the control series at the end of the twenty-six hour periods. This difference has been practically constant under these conditions, but an almost equally marked increase in mononuclear cells may be encountered after repeated injections of alkaline salt solution over a period of several days. The reduction in the total leucocyte count from 8,800 to 6,800, as shown in the hematin counts, is misleading, as I have found an increase in the leucocyte count more common than a decrease. This feature of the hematin reaction will be considered later.

The production of a chronic intoxication by daily injections of small doses of hematin for a week or more brings out distinctly the resemblance of the leucocyte picture of hematin intoxication to that of malaria. Following each dose of hematin there is a cycle of changes, such as is illustrated in table I, with the mononuclear cells dominating the picture more and more; especially is this true of the large mononuclears which gradually increase until they constitute from 18 to 25 per cent. of a leucocyte count that is normal, slightly increased, or rarely reduced. The tendency to a slight leucocytosis is still the one striking discrepancy between the two pictures. Pigmented macrophages are frequently present in large numbers and have been found in the blood as much as seventy-two hours after an injection of hematin. The polymorphonuclear leucocytes may also show phagocytosis although this is more common in extreme intoxications.

A typical example of the effect of chronic hematin intoxication upon the leucocytes is presented in table II, which contains the normal and final leucocyte counts made upon rabbit 15.

Rabbit 15.—Grey and white, male; weight, 1.6 kilos.

January 16, 1912. White blood corpuscles, 8,300.

This animal received 10 mg. of hematin per kilo of body weight on ten successive days.

January 27. White blood corpuscles, 11,800. The differential counts are shown in table II.

TABLE II.

The Leucocyte Reaction in Chronic Hematin Intoxication.

Time.	Leucocyte count.	Polymorphonuclear cells.		Small mononuclear cells.		Large mononuclear cells.		Eosinophiles.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Jan. 16, 1912, 8.30 A.M.	8,300	42.8	3,552	48.4	4,017	5.6	465	1.4	116	1.8	149
Jan. 27, 9.00 A.M.	11,800	23.8	2,808	50.2	5,924	22.2	2,620	0.4	47	3.4	401

If the conditions existing in pernicious forms of malaria be simulated by the injection of as much as twenty milligrams of hematin per kilo of body weight, and one or two injections be given daily,[2]

[2] Unless the animal is in the best condition it can not survive this dosage of hematin. The first injection will prove fatal to a few rabbits and the number of deaths will increase with successive injections, especially if the injections are given less than ten to twelve hours apart.

within a few days the animal will develop a marked leucocytosis. The polymorphonuclear amphophiles show a relative increase, the percentage of small mononuclears is decreased or normal, while the percentage of large mononuclears is normal or increased. Both the eosinophiles and basophiles are relatively decreased under these conditions. The most striking feature of the count is the high percentage of large mononuclear cells. While the percentage increase in these cells is not so great as in chronic intoxications it not infrequently reaches 12 or 14 per cent. of the total count. The normal and the final counts in an experiment of this character are given to illustrate this point.

Rabbit 19.—Grey, female; weight, 1.7 kilos.

March 18, 1912. White blood corpuscles, 11,400. Seven injections of 25 mg. of hematin, per kilo of body weight, were given between March 18 and 21, inclusive.

March 22. White blood corpuscles, 52,600.

TABLE III.

The Leucocyte Reaction in a Case of Extreme Hematin Intoxication.

Time.	Leucocyte count.	Polymorphonuclear cells.		Small mononuclear cells.		Large mononuclear cells.		Eosinophiles.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
March 18, 9.00 A.M.	11,400	36.8	4,195	47.6	5,426	7.0	798	3.0	342	5.6	638
March 22, 9.00 A.M.	52,600	62.2	32,710	20.6	10,835	15.0	7,890	1.0	526	1.2	631

It will be noted that the increase in large mononuclear leucocytes, as shown in table III, is relatively greater than the increase in polymorphonuclear cells. This is not usually the case and is, therefore, to be regarded as an extreme example of the reaction of the large mononuclear leucocytes to hematin.

On reviewing the changes in the leucocytes induced by the injection of alkaline hematin, the increase in the total count of these cells with the lesser degrees of intoxication seems to be the feature most at variance with the leucocyte picture of malaria A partial explanation of this difference is to be found in the fact that alkaline hematin is a strong local irritant and that it is extremely difficult to inject into an ear vein, even in dilute solution, without exciting a local inflammatory reaction, while thrombosis of the vein is certain to follow a limited number of injections into the same vessel. Although all animals that showed any considerable inflammatory reaction have been discarded, it seems possible that with the strength of alkaline hematin that it has been necessary to use, the irritant action has not been without effect. The degree of this effect is impossible of estimation.

As regards the most characteristic feature of the leucocyte reaction,—the increase in large mononuclear cells,—a portion of this increase is certainly attributable to the alkaline salt solution used as the solvent for hematin, but, when this is deducted, I believe there is still a substantial margin that can be accounted for only by the action of the hematin.

Platelets, Coagulation, and Bleeding Time.—The most striking effect of hematin upon the blood is its destruction of the platelets and consequent prolongation of the bleeding time. While the normal number of platelets in the rabbit varies between rather wide limits, it is usually between 600,000 and 800,000, estimated by the method of Wright and Kinnicutt,[3] and in a given animal it remains fairly constant from day to day. Following the injection of even a small dose of hematin, there is an immediate reduction in the number of platelets and the count continues to decrease for several hours. Large doses of hematin produce a correspondingly greater destruction of platelets which may exceed one half the total platelet count. If no further injections are given, the count will return to normal, or above, within twenty-four to forty-eight hours. Daily repetition of the injections of hematin, however, will greatly reduce the platelet count,—usually not lower than about one fourth of the normal count. A single dose of hematin sufficiently large to produce a greater reduction, or the repetition of smaller doses sufficiently often to accomplish the same result, are apt to prove fatal. In several instances, however, I have recorded greater reductions than this, the greatest being a reduction from 928,000 to 92,000. The series of counts in table IV is intended to illustrate the essential features of the effect of hematin upon the platelets as compared with the effect of alkaline salt solution.

[3] Wright, J. H., and Kinnicutt, R., *Jour. Am. Med. Assn.*, 1911, lvi, 1457.

TABLE IV.

The Effect of Hematin upon the Platelet Count.

Time after injection.	No. 34. 25 mg. of hematin per kilo.	No. 31. An equivalent volume of alkaline salt solution.
Normal	616,000	560,000
1 hr.	192,000	598,000
3 hrs.	232,000	740,000
5 hrs.	156,000	646,000
24 hrs.	312,000	608,000
48 hrs.	480,000	594,000
75 hrs.	788,000	622,000

In connection with the destruction of the platelets, a tendency to persistent and even profuse hemorrhage is of frequent occurrence. A series of observations made on the coagulation time with the Boggs coagulometer and on the bleeding time by the method of Duke[4] may be reported briefly. It was found that the ultimate effect of hematin was to prolong both the coagulation time and the bleeding time. The longest coagulation time recorded with the Boggs instrument was seventeen minutes, while eight to twelve minutes were not uncommon. In several instances, however, blood drawn in a test-tube remained permanently unclotted. The bleeding time is apparently much more affected. The ear vessels are usually abnormally constricted but if a normal degree of dilatation be procured profuse hemorrhage occurs from small cuts and bleeding continues for hours. In several extreme cases it was necessary to place a clamp on the ear after bleeding had continued two or three hours with no indication of cessation.

These phases of the action of hematin are apparently not influenced in the slightest degree by the presence of the alkaline salt solution. As regards the platelets, control experiments show almost uniformly an increase in the platelet count (table IV) after the injection of alkaline salt solution. Not a single instance of a definite decrease in the platelets has been observed. And no effect of any

[4] Duke, W. W., *Jour. Am. Med. Assn.*, 1910, lv, 1185.

kind has been noted upon either the coagulation or the bleeding time following the injection of alkaline salt solution.

DISCUSSION.

Comparing the various phases of the effect of alkaline hematin upon the blood of the rabbit with the blood in malarial fevers it can not be claimed that there is an absolute identity and yet there are no very great differences. The characteristic anemia of malaria has been ascribed to several accessory causes apart from the destruction of the red cells by the parasite developing within them. Prominent among these accessory causes has been the theory of a circulating toxin, destructive to the red cells. Hematin fulfills the essential conditions for such a circulating toxin, and it is only remarkable that this pigment should cause so pronounced an anemia as it has been found capable of producing. In this connection, it does not seem amiss again to call attention to the fact that the hemoglobin converted to hematin by the malarial parasite is not readily available for the regeneration of red cells, and the blood in this manner is rapidly depleted of hemoglobin iron. This condition undoubtedly contributes to the perpetuation of the malarial anemia.

Concerning the influence of hematin upon the leucocytes of malaria, one can speak with less assurance. In the first place, the possible normal variations of these cells in the rabbit are very great. Added to this is the influence of alkaline salt solution, which is difficult to estimate, and finally the difficulty of adjusting the dose of hematin so as to produce desired degrees of intoxication. Therefore only broad generalizations seem to be permissible. The leucocytosis of hematin poisoning is in sharp contrast to the leucopenia of the milder forms of malaria, but it coincides with the leucocytosis of the pernicious forms. The increase in the large mononuclear leucocytes, which is so characteristic of malarial blood, is also a prominent feature of the blood of hematin intoxication. Although the percentage of these cells is probably not as high as in malaria, this would hardly be expected as there are certainly other causes that contribute to the increase in large mononuclear leucocytes Finally, the general cycle of changes produced by an injection of hematin is, at least qualitatively, analogous to changes occurring in the blood with the paroxysm of malaria. Altogether, it would seem that hematin must play some part in the determination of the leucocyte picture of malaria.

Lastly, the destructive action of hematin upon the platelets and the consequent tendency to hemorrhage is not without a parallel in malaria. The platelets are probably the only bodies in the blood whose numbers in malaria are influenced solely by the circulating toxin of the disease, and it is of especial significance, therefore, that the platelets are reduced both in malaria and in hematin intoxication. The tendency to hemorrhage produced by hematin has its counterpart in the well recognized class of hemorrhagic cases of pernicious malaria. Here again the circulating hematin probably plays the important rôle in the production of the hemorrhagic condition. I do not wish to create the impression, however, that hemorrhages in malaria, or in hematin poisoning, are due solely to the destruction of platelets and prolongation of the bleeding time. This is certainly not the case in hematin poisoning in which profuse hemorrhage into the peritoneal cavity is of frequent occurrence in acutely fatal poisoning.

The pigment, hematin, must be regarded as an active factor in the production of many, if not all, of the important changes in the blood that characterize the various forms of malaria.

SUMMARY.

1. Intravenous injections of alkaline hematin in the rabbit produce an anemia the severity of which is proportional to the amount of hematin injected and the susceptibility of the animal.

2. Hemoglobinemia is an occasional consequence of hematin poisoning.

3. The leucocytes in hematin intoxication are usually increased in number and are always characterized by a high percentage of large mononuclear cells and by pigmented phagocytes.

4. The platelets are markedly reduced by alkaline hematin and ultimately a prolongation of the coagulation time of the blood and of the bleeding time results.

5. The anemia, the hemoglobinemia, the high percentage of large mononuclear leucocytes, the destruction of platelets, and the tendency to hemorrhage in malaria are all influenced by the malarial pigment, hematin.