MALARIAL PIGMENT (HEMATIN) AS A FACTOR IN THE PRODUCTION OF THE MALARIAL PAROXYSM.*

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It is remarkable that, from the enormous amount of investigation that has centered about the malarial parasite and its relation to malarial fevers, there has come no clear exposition of the mode of production of the various phenomena of the malarial paroxysm. It is true that these phenomena have been ascribed to the presence in the circulation of some toxic substance, or substances, elaborated by the malarial parasite, and that the blood at the time of the segmentation of the parasite has been shown to possess toxic properties. Still, as far as I know, the nature of these toxic agents remains unknown, as no one has clearly demonstrated the presence of any definite substance, which, when introduced into the circulation, could reproduce the symptom complex of the malarial paroxysm. The observations embodied in this report are offered, therefore, with the hope of shedding some light upon the question.

In the course of some work on hematin metabolism, it was noted that a rabbit that had received an intravenous injection of alkaline hematin developed a very pronounced shaking chill, strikingly like that of malaria. As the author has attempted to show in a previous paper,[2] the pigment elaborated from the hemoglobin of the red blood corpuscle by the malarial parasite and liberated into the circulation of the host at the time of segmentation of the parasite is undoubtedly hematin. It at once appeared possible, therefore, that we might find in this substance one of the hypothetical toxins operative in malaria.

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[1] The first seven experiments of this investigation were done in the Pathological Laboratory of the University of Wisconsin, Madison, Wis.

[2] W. H. Brown, *Jour. Exper. Med.*, 1911, xiii, 290.

The investigation of this question now embraces a series of ninety observations upon the effect of the intravenous injection of alkaline hematin, eighteen rabbits having been used for test purposes.

TECHNIQUE.

Materials Used and Their Preparation.—The hematin used was derived from three sources, rabbit blood, dog blood, and ox blood, but in all cases it was prepared by the Schalfijew process. The solutions for injection were made in 0.85 per cent. salt solution containing 1.5 or 2 per cent. bicarbonate of sodium. The strength of the hematin employed has varied from 1.5 to 5 milligrams per cubic centimeter. The hematin was added to the sterile solvent and heated to 100° C. for five to ten minutes, and was then allowed to stand for twelve to twenty-four hours, when it was again heated and, while still hot, filtered into sterile flasks. The subsequent treatment of these solutions has varied somewhat. Solutions prepared thus, and again heated to 100° C. for five to ten minutes have apparently remained sterile. In a few instances, however, the filtered solution has been autoclaved to insure sterility.

It has been found extremely difficult to prepare hematin solutions of absolutely uniform character. With a given preparation of hematin and with the same technique in the preparation of the solutions, two distinct types of solution may be obtained: one, a perfectly clear, deeply colored solution that even under the microscope shows very few particles of pigment suspension, and on standing shows no precipitation in the flask; the other, a turbid solution that appears chocolate brown in thin layers and under the microscope shows myriads of pigmented particles and droplets floating in an only faintly colored fluid. Much of this pigment is precipitated on allowing the solution to stand. This latter "colloidal" type of solution or suspension, manifests all the optical properties of an alkaline hematin solution and the suspended pigment readily passes through Schleicher and Schüll filter paper No. 597 which has been used as the routine filter. With different preparations of hematin these variations in solubility are continually appearing. Reference is made to this feature of the hematin solution to indicate the

difficulty in maintaining absolutely uniform experimental conditions and accurate dosage.

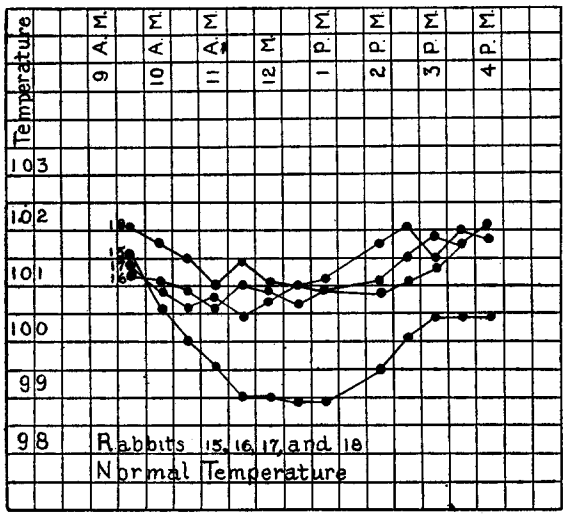
Experimental Procedure.—Briefly, the experiments have been conducted according to the following plan: The animals were accustomed to laboratory surroundings by being kept in cages from one to two days before any observations were made. The normal temperature curve of each animal was then established by rectal temperature, taken every half hour from 8 or 9 A. M. until 3 or 4 P. M., the time to be covered by subsequent experiments. As it seemed probable that the sodium bicarbonate salt solution used as the solvent for hematin might produce some toxic manifestations when injected intravenously, the action of this solution was determined in a number of instances, usually in such doses as were subsequently to be administered with the hematin. While these control tests were usually made prior to the injection of hematin, some such tests followed the administration of hematin. The animals were next given intravenous injections of alkaline hematin at 8 or 9 A. M., and the resulting phenomena were noted, the temperature being taken as previously, although fifteen minute observations were frequently made.

Dose of Hematin.—It is evident that the question of dosage in such a series of experiments is one of prime importance. To carry out the object of these experiments it is quite essential that the dose of hematin employed be somewhat comparable to that liberated into the human circulation at the time of segmentation of a generation of parasites. While there is no evidence to show that all the hemoglobin of the infected red corpuscles is decomposed to form hematin, if we may assume that such is the case, and, further, that a 1 to 5 per cent. infection of red blood corpuscles is not uncommon, we have sufficient data upon which to base a calculation of the approximate amount of hematin liberated into the human host with the segmentation of a given generation of parasites. Basing our calculations on the presence of 8.5 grams of hemoglobin per kilo of body weight, and 4.47 per cent. of hematin in hemoglobin,[3] we find that in a 1 per cent. infection of the red blood corpuscles approximately

[3] Olof Hammarsten, A Text Book of Physiological Chemistry, translated by Mandel, New York, 1901, p. 139. These figures are probably high.

3.7 milligrams of hematin per kilo of body weight would be liberated. In my experiments the dosage has varied from 1.3 milligrams to 28 milligrams per kilo of body weight, given in single or in divided doses, corresponding roughly to an infection of 0.3 to 7.5 per cent. of the red blood corpuscles.

Normal Temperature of the Rabbit.—In undertaking an investigation which must necessarily deal so largely with variations in the temperature of the experimental animal, it is imperative that the basis of comparison between normal and abnormal fluctuations of temperature be as free from objection as possible. Unfortunately, the temperature of different rabbits varies widely, even when kept under exactly the same conditions. In apparently normal animals I have found the individual extremes between 98° and 103° F. Likewise, the fluctuations of temperature in a given animal may be quite considerable, but usually follow a fairly definite course.



TEXT-FIG. 1. Normal temperature of four rabbits during the period of the experiments.

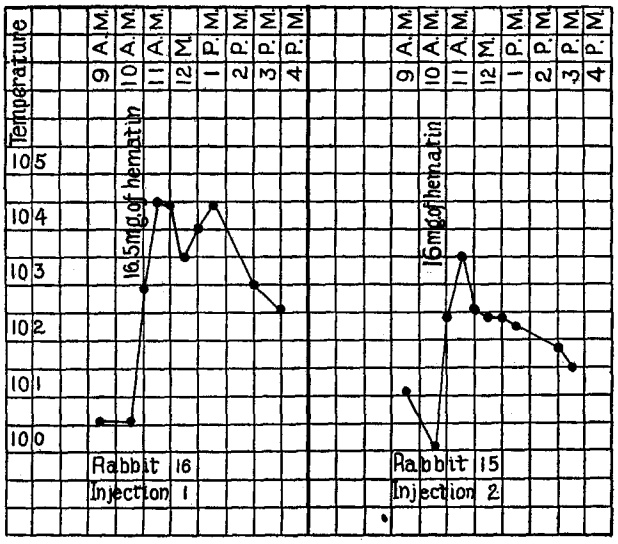
The course and fluctuations of temperature of the normal rabbit under experimental conditions, as well as the individual difference in temperatures, are shown in text-figure 1. This chart shows the normal temperatures recorded for rabbits 15, 16, 17, and 18. The

first three animals were from the same litter, about three quarters grown, and weighed 1,600 to 1,700 grams. Number 18 was full grown and weighed 1,840 grams. All the records were taken at the same time and all conditions were as nearly alike as possible. While three of these curves coincide closely, the fourth shows an extremely low and irregular curve of temperature. It should be noted that the temperature in all instances has a downward trend during the morning hours, and does not show an upward tendency until about noon, when there is a gradual rise, which ultimately reaches as high as the temperature at the first observation or even higher. This temperature curve has been fairly constant in my entire series of experiments.

Effect of Hematin upon Temperature.—If, for purposes of comparison, we adopt the classical division of the malarial paroxysm into a cold stage, a hot stage, and a stage of sweating, with the concomitant symptoms belonging to each, certain of these manifestations are capable of accurate measurements in an experimental animal, while others may be determined with a fair degree of accuracy by close observations, and still others are wholly indeterminable. Of prime importance among these phenomena of the malarial paroxysm is the question of fever.

In estimating the temperature effects, in all instances at least three facts are to be taken into consideration; the nature of the effect, the degree of the effect, and the duration of the effect. While it has been possible to assemble much of the data concerning the effects of hematin upon the temperature in an appended table which shows the abbreviated protocols of the entire series of experiments, it must be fully appreciated that such tabulations of statistics are wholly inadequate to present many features of the experiments that are equally as important as those thus presented, and attention will be especially directed to such features. Further, as can be seen from these tables, it has been the object of the author to study effects in individual animals with a variety of doses, as occasion suggested, rather than to mould all the experiments to a single type or plan, for it became evident early in this investigation that individual peculiarities of the animals played a prominent rôle in the results obtained.

Without exception, every dose of hematin administered has elicited a definite temperature response. With but three exceptions, this response has been characterized by a sharp rise in temperature, reaching the fastigium in about an hour and a quarter. The further course was somewhat variable, although in most cases with a dose of 15 milligrams, or less, per kilo, there was a rather sharp fall of temperature for thirty minutes to one hour, followed by a secondary rise of variable extent and duration or a very gradual decline requir-



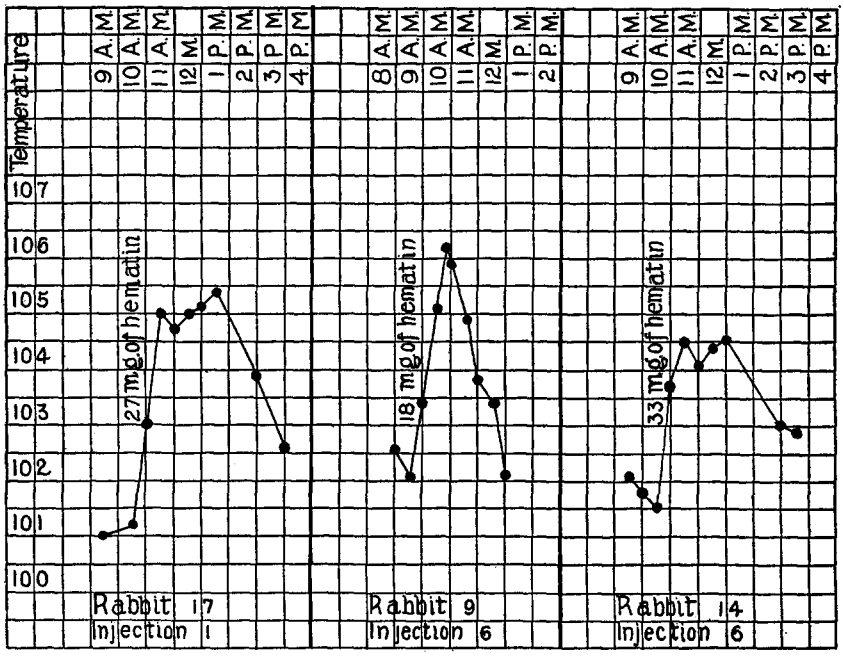
TEXT-FIG. 2. The usual types of temperature curve following injections of hematin.

ing several hours to reach normal again. Two such temperature curves are shown in text-figure 2.

These curves are subject to innumerable variations depending upon the dose, the stage of the experiment, and upon the individual peculiarities of animals. Some of the more important variations are an accentuation and prolongation of the secondary rise, usually shown with initial and large doses, or a defervescence that is almost as sharp as the rise in temperature. A third variation, which includes the three exceptions previously noted, is met with in instances

of marked intoxication and is characterized by an initial drop in temperature with a subsequent rise. All three of these modifications are illustrated in text-figure 3.

The extent of the temperature elevation is, within certain limits, proportional to the amount of hematin injected. The temperature effect, being very slight with small doses, increases with the dose,



TEXT-FIG. 3. Variations in the temperature curve following large doses of hematin or repeated injections of hematin.

until we begin to obtain signs of an over-intoxication when the elevation may be much less than with smaller doses, the optimum dose usually being between 10 and 15 milligrams per kilo of body weight. The elevation of temperature obtained with such optimum doses is from 3° to 3.5° F., and it is exceptional that a greater rise is reached. Occasionally, however, the temperature may rise 4° F. or even higher in highly susceptible animals. The greatest elevation recorded in my series of experiments was 4.9° F. in animal 9, with a dose of 18 milligrams of hematin per kilo.

In well-marked reactions, the temperature usually returns to within the normal range in the course of three to five hours. With large or initial injections of hematin, the period of elevated temperature is more prolonged than under other circumstances and seldom reaches normal in less than four hours, occasionally requiring as much as six hours. Exceptionally, the return to normal may be rapid (text-figure 3).

The method of administration also plays an important part in the results. A given dose of hematin injected in two or three fractional doses at intervals of fifteen to thirty minutes produces a much more marked elevation of temperature than when given at a single injection, an effect that is well shown in rabbit 13. This is particularly true of the smaller, or optimum doses, while with larger doses the increased potency may be manifested by a slowing of the rise of temperature, a cessation of the rise, or even a fall upon the injection of the second fraction, as illustrated in rabbit 12.

Neither the source of the hematin nor the type of solution seems to play an important part in the results that I have obtained. That is, there has been but slight difference between the action of rabbit, dog, or ox hematin, or between the action of the perfectly clear hematin solution and that containing much finely divided hematin in suspension.

However, a few tests seem to indicate that solutions of hematin gradually lose their pyrogenic properties with age or when subjected to high and prolonged temperature. This apparent decrease may be seen by comparing the results obtained in rabbit 7, injection 3, and rabbits 8, 10, and 11. Further, it can readily be imagined that all animals will not be found equally susceptible to the toxic action of hematin. A few will exhibit a marked sensitiveness and a few will be found extremely resistant, the optimum dose in the one producing but slight effect in the other. This variation in susceptibility was strikingly illustrated by animals 13 and 14 which were under observation at the same time. Rabbit 13 was a typically resistant animal, and rabbit 14 was highly sensitive.

Effect of Sodium Bicarbonate Salt Solution upon the Temperature.—Animals injected with the bicarbonate salt solution alone, for purposes of control, almost invariably showed an elevation of tem-

perature in proportion to the size of the dose, and about one third to one half the elevation produced with an equal amount of hematin solution, depending somewhat upon the concentration of the hematin in the solution. With small doses of the control medium, the fluctuations of temperature were usually within what might be termed the normal range and were such that it is difficult to say whether they are more than incidental to the process of injection. Large doses may produce a rise in temperature corresponding approximately to the over-intoxicating effect of large doses of hematin. In such instances, however, other features of the clinical picture will distinguish sharply between the two cases. In all instances, therefore, there were distinct differences between the action of the sodium bicarbonate salt solution and the action of the hematin solution, such that there can be no question as to the part played by the hematin in these experiments.

Other Phenomena of the Hematin Paroxysm.—Apart from the elevation of temperature in the experimental animal, the paroxysm of hematin intoxication presents other features which are of equal importance and show a strong resemblance to corresponding phenomena of the malarial paroxysm. For the first fifteen to twenty minutes following the injection of hematin, the rabbit usually manifests a slight degree of restlessness, then crouches in a corner of the cage. In the second stage of the paroxysm the vessels of the ears contract giving to the shaved ears a pale and cyanotic hue, while at the same time the ears become decidedly cold. In pronounced cases the surface temperature (temperature of the ears) may be more than 30° F. below the rectal temperature. The lowest temperature recorded in this series of experiments was 63.5° F. with a room temperature of 62.5° F. and a rectal temperature of 105° F. During this stage the animal's ears usually lie on its back, and the hair tends to become erect, presenting the picture of an animal that is cold. Meanwhile, the rabbit shows convulsive tremors or shivering, but rarely any continued or pronounced shaking. This stage of chill lasts from forty-five minutes to one hour, and is terminated rather abruptly by a dilation of the superficial vessels, the ears rapidly becoming flushed and hot. The animal now moves about the cage or stretches out and remains quiet. Further than this, the

third or hot stage of the paroxysm possesses no especial symptoms and its limit can be fixed only by the course of the temperature, which may remain well above normal for several hours, or sink to normal within an hour. During the third stage and the latter part of the second stage of the paroxysm the animal shows a pronounced thirst which is undoubtedly referable to the febrile condition.

The most striking and at the same time the most constant of all these symptoms are the contraction and dilatation of the superficial vessels associated with the corresponding lowering and elevation of the surface temperature.

The contrast between the symptoms of the animal injected with hematin and those of control animals is quite as sharp as in the instance of the temperatures. With doses of hematin sufficiently large to produce pronounced symptoms of the type described, corresponding doses of sodium bicarbonate salt solution produce practically no recognizable effect. There may be a suggestive or very transient change in the surface vessels and the temperature, but nothing that is definite or constant. When, however, larger doses, *e. g.,* ten cubic centimeters per kilo, are given, phenomena simulating the picture of hematin intoxication may be elicited, but the changes are not so definite or constant.

If now we correlate these symptoms with the temperature curve, we find that the elevation of temperature during the first stage is slight, and that the second stage of the paroxysm corresponds closely with the period of rising temperature, the initial drop coinciding sharply with the vascular dilatation and flushing of the ears and the elevation of the surface temperature. The third or hot stage, as previously noted, corresponds to the duration of the temperature above normal.

As in the case of the temperature, all other phenomena of hematin intoxication seem to be exaggerated when a given dose of hematin is divided into several fractional doses, the cycle of phenomena following closely consequent changes in the temperature curve.

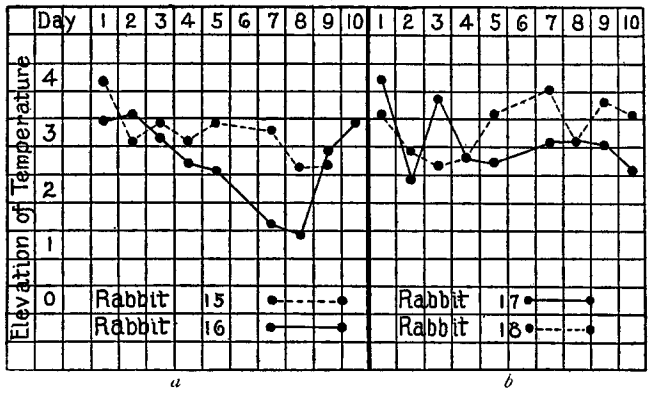
It must be pointed out, however, that the prominence of these paroxysmal phenomena and the degree of elevation of the temperature are by no means always parallel. The toxic paroxysmal phenomena may be present to a high degree in an animal that shows

only a slight elevation of temperature, and in most instances such a condition is to be regarded as evidence of over-intoxication.

Acquired Resistance.—Early in the course of these experiments it became evident that repeated injections of a given dose of hematin in the same animal did not give uniform results. The results, however, were of such a nature as to suggest that the animal acquired a certain degree of tolerance which, in turn, might be broken when the intoxication was pushed sufficiently. To determine this point the following experiment was carried out.

Experiment.—Four rabbits, weighing respectively 1,600, 1,650, 1,790, and 1,840 grams, were injected on ten successive days with a solution of ox hematin containing 5 mg. of hematin to 1 c.c. The first two animals received 10 mg. of hematin per kilo of body weight, and the other two received 15 mg. per kilo. Rectal temperatures were recorded every half hour.

The results are shown in text-figure 4. In *a* is shown the differences of elevation of temperature on successive days of the two



TEXT-FIG. 4. Variations in the elevation of the temperature with repeated injections of a given dose of hematin.

animals receiving ten milligrams per kilo, and in *b* those receiving fifteen milligrams per kilo.

While the curve of temperature reaction in each case is extremely irregular, it is in general characterized by a tendency towards a decrease, which in the instance of animals 16 and 17 persists throughout the experiment. With animals 15 and 18, however, a second phase of increased reaction is developed. These animals also

exhibited the most marked symptomatic effects throughout the experiment. If the temperature curve alone be considered, it is certain that the tendency is toward a decreasing response to successive injections of hematin and this I have found to be true in other experiments. If we take into consideration other evidence of intoxication, however, as in animals 15 and 18, this decrease seems referable not so much to a tolerance as to over-intoxication. Again, as these symptoms of intoxication decrease and the fluctuations of temperature increase correspondingly, there may be developed a certain degree of tolerance. On the other hand, as shown in animals 16 and 17, there may be an increasing resistance to the hematin from the start as the toxic symptoms as well as the temperature decrease proportionally.

This subject of acquired tolerance has been taken up largely to emphasize the importance to be attached to results obtained from properly adjusted initial doses of hematin but also to explain the apparent discrepancies in the results from any series of hematin injections. It is the initial injection, with but few exceptions, that gives the maximum temperature reaction obtainable with a given dose of hematin until the series of injections has been extended to such a degree as to permit of the acquirement of a tolerance in highly susceptible animals or to cause a break in the early acquired tolerance of more resistant animals. When such conditions supervene, the temperature reaction may again increase and show an even greater response than with the initial injection (table I, animals 9 and 18).

SUMMARY.

The paroxysm of hematin intoxication in the rabbit undoubtedly presents many features of striking similarity to the paroxysm of human malaria; still one must hesitate to apply such results unreservedly in an attempt to identify the causative agent of the malarial paroxysm. When, in addition to the character of the paroxysm, we consider the sequence of events in the two instances, the analogy becomes so close that it seems impossible to regard the matter as a mere coincidence. The injection of hematin, especially in fractional doses, is in a measure comparable to the liberation of hematin into the human circulation by the malarial parasite. In these experi-

TABLE I.

Summary of Experimental Data.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 1. Weight,	Jan. 27, 1911	5.0			102.3	1.1		2	102.6	Rabbit hematin in all injections.
2.49.	Jan. 30, 1911		5.0	3.0	102.5		2.5	3	105.0	Highest temperature on secondary rise.
	Jan. 31, 1911		10.0	6.0	101.8		2.2	1½	102.8	
	Feb. 1, 1911		5.0	3.0	103.0		1.4	1½	103.0	
	Feb. 2, 1911		20.0	12.0	103.0		0.0		96.4	In two doses, 5 minutes apart. Died 3½ hours after injection.
No. 2.	Feb. 1, 1911	10.0			102.8	1.8		2½	104.3	
Weight,	Feb. 2, 1911	10.0			102.2	1.8		1½	103.0	Rabbit hematin.
2.78.	Feb. 3, 1911	23.0			102.1	2.5		1	102.8	
	Feb. 7, 1911		5.0	2.6	102.9		3.1	1¼	106.0	Initial rise to 106° with fall to 105.3° F. and second rise.
	Feb. 8, 1911		2.5	1.3	102.6		1.2	1	103.0	
	Feb. 9, 1911		7.5	3.9	102.5		2.6	1¼	104.0	
	Feb. 11, 1911		7.5	3.9	102.4		3.0	1¼	103.9	
	Feb. 15, 1911		15.0	7.8	102.3		4.0	1¼	106.1	
	Feb. 16, 1911		20.0	10.4	102.8		3.3	1¼	104.9	
No. 3. Weight, 2.9.	Feb. 8, 1911		2.5		102.7					2.5 c.c. thick suspension of hematin in 0.85% salt solution. Died instantly.
No. 4.	Feb. 8, 1911	10.0			102.6	1.4		1½	102.8	
Weight,	Feb. 9, 1911		7.5	4.2	102.2		2.6	1¼	104.6	Rabbit hematin.
2.65.	Feb. 11, 1911		7.5	4.2	102.3		2.8	1¼	103.8	
	Feb. 15, 1911		10.0	5.7	102.4		2.5	1¼	104.8	

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours After Injection.	Remarks.
No. 4. Weight, 2.65.	Feb. 16, 1911		15.0	8.4	101.6		2.1	3	103.7	Temperature dropped to 100.7° F., then rose very slowly.
No. 5.	May 1, 1911	7.0			101.8	1.3		1½	102.2	
Weight,	May 2, 1911		7.0	5.0	102.0		3.6	1½	105.0	Dog hematin.
2.85.	May 3, 1911		7.0	5.0	102.3		3.2	1¼	104.7	
No. 6.	May 1, 1911	8.0			102.4	1.6		1½	103.0	
Weight,	May 2, 1911	10.0			101.8	1.2		1½	102.2	Other manifestations very
2.57.	May 3, 1911	15.0			102.0	1.7		1¼	103.3	slight.
	May 4, 1911	20.0			102.1	2.1		1½	103.5	
No. 7.	May 1, 1911	8.0			101.6	1.5		2	102.0	
Weight,	May 2, 1911		8.0	4.7	101.2		3.7	1¼	104.0	Rabbit hematin.
2.53.	May 3, 1911		8.0	6.3	101.3		3.1	1¼	103.6	Dog hematin.
No. 8.	Oct. 18, 1911	6.0			102.1	1.4		1½	102.8	Hematin solution 5 months
Weight,	Oct. 20, 1911		6.0	10.0	102.3		1.8	1½	103.0	old, same as that used in
1.85.										in No. 7, injection 3.
No. 9.	Oct. 20, 1911	12.0			102.5	2.0		1½	102.7	
Weight,	Oct. 23, 1911		12.0	12.0	102.3		3.3	1	103.4	Ox hematin in all injections.
2.05.	Oct. 25, 1911		12.0	12.0	102.5		2.7	1½	102.7	
	Oct. 26, 1911		12.0	12.0	103.6		2.0	¾	103.0	An afternoon experiment.
	Oct. 27, 1911		12.0	12.0	102.1		3.7	1	103.1	Temperature back to 103.6°
	Nov. 1, 1911		12.0	12.0	102.1		4.1	1¼	103.4	in 1½ hours.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 9.	Nov. 3, 1911		12.0	12.0	101.8		4.3	1½	——	
Weight,	Nov. 6, 1911		18.0	18.0	101.5		4.9	1	103.5	Given in two doses 5 minutes apart.
2.05.										
No. 10.	Oct. 25, 1911		12.0	20.0	102.6		0.8		103.0	Hematin solution 5½ months old, same as No. 7, injection 3, and No. 8, injection 2.
Weight,	Oct. 27, 1911		12.0	20.0	102.2		1.5		102.8	
1.85.										
No. 11.	Oct. 27, 1911		5.0	7.5	102.6		0.6	1	102.5	Hematin solution, same as No. 10.
Weight,	Nov. 3, 1911	12.0			101.6	1.3		1½	101.6	
2.0.	Nov. 6, 1911	18.0			101.0	2.4		2	102.2	Ox hematin in all other injections.
	Nov. 8, 1911		6.0	6.0	101.4		2.6	1	102.8	
	Nov. 10, 1911		12.0	12.0	100.4		3.0	1¾	102.8	
	Nov. 13, 1911		18.0	18.0	100.3		3.9	3	104.2	
	Nov. 15, 1911		10.0	20.0	100.5		4.5	1½	103.0	
	Nov. 17, 1911		8.0	16.0	100.8		3.2	1¼	103.2	
No. 12.	Nov. 10, 1911		15.0	15.0	101.2		3.2	1½	103.9	Ox hematin.
Weight,	Nov. 13, 1911		18.0	18.0	101.3		4.0	1½	105.0	
2.0.	Nov. 15, 1911		10.0	20.0	101.0		3.3	1	102.9	
	Nov. 17, 1911		8.0	16.0	101.7		3.1	1¾	104.0	
	Nov. 20, 1911		10.0	20.0	102.5		1.9	1½	103.3	In two doses 30 minutes apart. Initial rise checked by second dose. Apparently about the optimum dose.
	Nov. 22, 1911		5.0	10.0	101.4		3.4	1¼	103.4	
	Nov. 24, 1911		4.0	8.0	101.5		4.2	1¼	104.0	
	Nov. 27, 1911		8.0	16.0	101.4		3.0	1½	103.1	

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 13.	Nov. 22, 1911	5.0			102.2	0.4		1	101.8	Ox hematin.
Weight,	Nov. 24, 1911		4.3	10.0	102.0		1.5	1	102.2	Single dose.
2.15.	Nov. 27, 1911		4.3	10.0	102.2		3.3	1¼	102.5	In 2 doses 30 minutes apart.
	Nov. 29, 1911	4.3			102.2	1.4		1¾	102.7	In 2 doses 30 minutes apart.
	Dec. 4, 1911	4.3			101.6	1.4		2¾	102.8	In 2 doses 30 minutes apart.
	Dec. 6, 1911		4.3	10.0	101.8		3.0	1¼	103.6	In 2 doses 30 minutes apart.
	Dec. 8, 1911		2.1	5.0	101.7		2.1	1¾	102.6	In 2 doses 30 minutes apart.
	Dec. 11, 1911		10.0	23.0	101.5		2.9	1¼	103.8	In 2 doses 30 minutes apart.
	Dec. 13, 1911		10.0	23.0	102.1		2.6	1	103.0	Single dose.
	Dec. 15, 1911		12.0	28.0	101.5		3.5	1	103.5	Single dose.
	Dec. 18, 1911		12.0	28.0	101.4		4.3	1½	104.4	Given in 2 doses 30 minutes apart.
										Ox hematin.
No. 14.	Dec. 6, 1911	4.5			100.7	1.9		2¼	102.2	Given in 2 doses 30 minutes apart.
Weight,										
2.25.	Dec. 8, 1911	2.2			100.4	1.6		2	——	Given in 2 doses 30 minutes apart.
	Dec. 11, 1911		2.2	5.0	101.0		3.2	1¾	103.4	Given in 2 doses 30 minutes apart.
	Dec. 13, 1911		3.3	7.5	101.4		3.4	1¾	103.6	Given in 3 doses 30 minutes apart.
	Dec. 15, 1911		4.5	10.0	101.5		2.6	1½	103.0	Given in 2 doses 30 minutes apart. Over-intoxication.
	Dec. 18, 1911		6.6	15.0	101.7		2.8	1½	104.5	Single dose. Over-intoxication.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours After Injection.	Remarks.
No. 15.	Jan. 12, 1912	3.2			100.6	1.5		1½	100.6	
Weight,	Jan. 16, 1912		3.2	10.0	99.8		3.2	1½	102.5	Ox hematin.
1.6.	Jan. 17, 1912		3.2	10.0	100.1		3.4	1½	102.3	
	Jan. 18, 1912		3.2	10.0	101.0		3.0	1	101.8	
	Jan. 19, 1912		3.2	10.0	101.2		2.5	1	102.6	
	Jan. 20, 1912		3.2	10.0	101.0		2.4	1½	101.9	
	Jan. 21, 1912									Another injection here without a record of temperature.
	Jan. 22, 1912		3.2	10.0	101.6		1.5	1½	102.0	
	Jan. 23, 1912		3.2	10.0	101.5		1.2	2½	102.7	Initial fall in temperature.
	Jan. 24, 1912		3.2	10.0	101.5		2.7	1	103.3	
	Jan. 25, 1912		3.2	10.0	101.8		3.2	1	104.0	
No. 16.	Jan. 12, 1912	3.3			101.1	2.7		1½	102.3	
Weight,	Jan. 16, 1912		3.3	10.0	100.6		3.9	1	104.4	Ox hematin.
1.65.	Jan. 17, 1912		3.3	10.0	101.8		2.8	1	102.9	
	Jan. 18, 1912		3.3	10.0	102.1		3.1	1	101.8	
	Jan. 19, 1912		3.3	10.0	102.0		2.9	1½	103.3	
	Jan. 20, 1912		3.3	10.0	102.5		3.2	1½	103.0	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		3.3	10.0	102.0		3.0	1½	103.1	
	Jan. 23, 1912		3.3	10.0	102.1		2.6	1½	103.0	
	Jan. 24, 1912		3.3	10.0	102.3		2.4	1	——	Leg broken by accident. Discontinued.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 17. Weight, 1.79.	Jan. 12, 1912	5.4	5.4	15.0	101.2	2.5		$1\frac{1}{2}$	103.2	Ox hematin.
	Jan. 16, 1912				101.3		4.0	3	105.3	Temperature reached 105° F. in 1 hour.
	Jan. 17, 1912		5.4	15.0	101.8		2.2	1	102.7	
	Jan. 18, 1912		5.4	15.0	101.5		3.6	1	102.6	
	Jan. 19, 1912		5.4	15.0	102.0		2.6	$1\frac{1}{2}$	103.3	
	Jan. 20, 1912		5.4	15.0	102.0		2.6	$1\frac{1}{2}$	103.3	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		5.4	15.0	102.2		2.8	$1\frac{1}{2}$	103.2	
	Jan. 23, 1912		5.4	15.0	102.1		2.9	$1\frac{1}{2}$	103.3	
	Jan. 24, 1912		5.4	15.0	102.6		2.8	1	103.3	
	Jan. 25, 1912		5.4	15.0	102.6		2.6	1	103.1	
No. 18. Weight, 1.84.	Jan. 12, 1912	5.5			101.5	2.6		2	103.2	
	Jan. 16, 1912		5.5	15.0	101.6		3.4	3	105.0	Ox hematin.
	Jan. 17, 1912		5.5	15.0	102.0		2.8	$1\frac{1}{2}$	103.5	
	Jan. 18, 1912		5.5	15.0	101.9		2.5	1	103.6	
	Jan. 19, 1912		5.5	15.0	102.3		2.6	$1\frac{1}{2}$	103.8	
	Jan. 20, 1912		5.5	15.0	101.5		3.4	$1\frac{1}{2}$	103.7	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		5.5	15.0	101.9		3.8	1	103.4	
	Jan. 23, 1912		5.5	15.0	102.2		2.8	$1\frac{1}{2}$	104.7	
	Jan. 24, 1912		5.5	15.0	102.5		3.6	1	103.7	
	Jan. 25, 1912		5.5	15.0	102.7		3.3	1	104.6	

ments, both solution and finely divided suspensions of hematin have been found equally effective in eliciting the phenomena of the paroxysm, and while it seems possible that a portion of the malarial pigment might be dissolved in the alkaline human serum, such an assumption is probably not essential.

It might be objected that the toxic action of foreign hematin thus injected into the circulation would probably be greater than that of hematin derived from an animal's own blood, but as far as I have been able to determine, this objection does not seem valid, as rabbit hematin, dog hematin, and ox hematin produce in the rabbit effects that are alike in both character and degree.

The dose of hematin remains as the one factor to which it is possible to attach some degree of uncertainty, but even here the author feels that the range of experimental conditions has been kept within the bounds of legitimate analogy with conditions existing in the human subject of malarial infection.

Finally, the most conservative estimate of the value of such experiments points strongly to the fact that we have at least a potentially toxic substance in the pigment hematin as liberated by the malarial parasite into the circulation of the human host.

There is also abundant evidence to show that the action of hematin is not confined to the paroxysmal phenomena of malaria, but that other features of the disease may find their explanation in the action of this pigment. For the present, however, it seems advisable to confine the discussion to this one phase of the question.

CONCLUSIONS.

1. Alkaline hematin in doses commensurate with the amounts of hematin liberated in the human circulation by the segmentation of the malarial parasite, produces, when injected intravenously into the rabbit, a paroxysm which is characterized by a short prodromal stage, a stage of chill and rising temperature, and a hot stage. In their details the phases of this paroxysm are practically identical with the corresponding ones in the paroxysm of human malaria.

2. The phenomena in human beings infected with malaria are, at least in part, directly referable to the toxic action of this malarial pigment.