HENRY'S TEST IN MALARIA: THE NATURE AND CLINICAL VALUE OF THE REACTION.

by

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C O N T E N T S.

I.

INTRODUCTION.

1.

The thesis here presented is based on the results of an investigation into the subject of Henry's serum precipitation reaction in malaria. The history of the reaction is traced from its introduction by Henry, to the recent improvements in technique elaborated by Greig, van Rooyen and Hendry, and to the further observations carried out by the present writer. The method of preparing the solution used for the test is detailed, and the mode of performing it is described. The nature of the reaction is discussed at some length, with particular reference to the part played by the melanin itself, while former views and titles for the reaction are critically reviewed. Then follows the detailed results of the actual tests carried out by the writer. These are compared with those obtained by other workers, and finally, conclusions are drawn as to the value of the reaction in the diagnosis of malaria. The investigation has called for research into other laboratory findings in malaria, and the results obtained are incorporated in the paper. A complete list of references is appended.

2.

The work was carried out in the Hospital for Tropical Diseases, London, between the months of June and October, 1934.

3.

The writer is grateful to Dr. G.Carmichael Low, Dr. Philip Manson-Bahi, and Dr. N. Hamilton Fairley, for permission to use their cases; to Mr. R.J. Bromfield for aid in the laboratory; to Mr. C. Naunton Morgan, Surgeon to the Metropolitan Hospital, London, for allowing him to have sera from cases attending the Venereal Diseases Clinic; and to Dr. G.M. Findlay of the Wellcome Bureau of Scientific Research, London, for furnishing him with samples of serum from guinea pigs suffering from experimental trypanosomiasis; he is deeply indebted to Lieut.-Colonel E.D.W. Greig, Dr. C.E. van Rooyen, and Mr. E.B. Hendry, of Edinburgh University and Edinburgh Royal Infirmary, for so kindly furnishing all the melanin solution used in the test, and to Dr. C.E. van Rooyen for demonstrating the test to him personally.

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II.

THE SERUM PRECIPITATION REACTION IN MALARIA.

1. HISTORICAL.

In 1927, A.F.X. Henry, in a paper presented to the "Association Française pour l'advancement des Sciences" at Constantine, described a new serological test for the diagnosis of malaria. For some time previous to this he had been considering the Wassermann reaction in syphilis, and at the same time his interest had been aroused by a statement occurring in Klesch and Kleiner's "Handbook on Tropical Diseases", and reading as follows:- "All histo-pathological lesions in malaria are occasioned by the deposit in the organs of two pigments, the brown iron containing pigment, and the black pigment, the so-called melanin pigment, whose composition is not fully understood." Henry (1927) came to the conclusion that these pigments were not merely deposits, but were active substances - in fact, malarial endogens which either produced anti-endogens or so disturbed the colloidal state that certain flocculating peculiarities of the serum resulted. Experimenting on the basis of these conclusions he found that the sera of malarial patients flocculated a solution of iron methyl-arsenate and a solution of melanin pigment. The former

reaction he termed ferro-flocculation, and the latter melano-flocculation. The ferro-flocculation reaction was found to be not absolutely specific for malaria, so its further investigation was abandoned. On the other hand the melano-flocculation reaction proved to be specific, and considerably more sensitive - a fact confirmed by Pozzi (1930). Henry concluded that the reaction was due to the action of the malarial parasite on the red cells, whereby the parasite destroying the red corpuscle transforms the haemoglobin into melanin, and at the same time forms an ochre pigment which accumulates in the liver; but Chorine and Gilliet (1933) found by experiments that neither melanin nor ochre pigment produces a positive Henry reaction in rabbits.

Several other continental workers, including Le Bourdelles and Liégois (1929), Biasiotti (1933), Sergent (1933), Gerbinis (1934) and Voigtlander (1934), have conducted investigations on Henry's reaction, while Greig, Rooyen and Hendry (1934) in this country have recently published the results of their work on the subject. The last-named observers improved on Henry's original technique, and it is their method which has been employed in these investigations.

2. RECENT IMPROVEMENTS IN TECHNIQUE.

Henry used melanin pigment derived from ox choroid membranes as "antigen". Later Livierato, Vagliano and Constantakato (1932) employed "antigen" prepared from the "ink sac" of a cuttlefish, while Tzechnowitza, Gorchowa and Moldavaskaja-Kritschewskaja (1932) worked with choroid of rabbits' eye. Greig, van Rooyen and Hendry found that crude aqueous suspensions of ox choroid were unstable, and in addition contained too large a proportion of ox tissue derivatives other than the melanin pigment itself. Consequently human hair has been introduced by these workers as a source for the extraction of melanin pigment. Briefly, this is obtained by the hydrolysis of the hair with concentrated hydrochloric acid, followed by distillation *in vacuo*, dialysis of the extract through a collodion membrane, and finally a concentration to represent a fixed amount of pigment.

3. MODE OF PREPARATION OF SOLUTION USED.

The details of the preparation of the melanin extract from hair as described by the above-named observers are as follows:- "25 grammes of human hair are refluxed with 100 ccs. of 50 per cent hydrochloric acid (by volume) for

5 to 6 hours. The dark brown hydrolysate is filtered through paper and the black insoluble residue discarded. It appears that the darker the hair, the greater the amount of insoluble humin matter it contains, and from a jet-black sample, a relatively poor yield of soluble pigment is obtained. The hydrolysate is then distilled *in vacuo* to remove the bulk of the volatile acid. At least three distillations should be made, water being added each time as soon as the residue becomes pasty. This residue is completely soluble in distilled water giving a very dark brown solution. The remainder of the hydrochloric acid and a considerable portion of the amino-acids present are removed by dialysing the mixture in a collodion sack against running tap-water. After 6 to 10 hours, depending upon the completeness of the vacuum distillations, the solution is only slightly acid to litmus paper and dialysis is then stopped. The mixture inside the sack is transferred to a 4 in. porcelain basin and 10 per cent sodium hydroxide solution is added until the reaction is slightly alkaline to litmus. The bulk of the precipitate should dissolve on this treatment but warming on the steam-bath may be necessary. Any remaining precipitate is removed by filtration. In all cases a dark brown, water-clear solution

should be obtained. It may be sterilised by transferring into test-tubes which are heated in a boiling water bath for 20 to 30 minutes, and sealed off as soon as they are removed. The colloidal solution of melanin so prepared appears to keep indefinitely. It does not give a positive ninhydrin reaction, is completely free from iron, but contains some compound of sulphur which gives a positive nitro-prusside reaction after fusion with sodium. This solution may be compared colorimetrically against a solution containing 10 grammes of ferric alum per 100 ccs. of distilled water. Assuming that a solution giving the same depth of colour as the standard represents 1 unit of melanin per 100 ccs., then a stronger solution representing about 4.8 to 5.0 units per cent appears to be the most satisfactory. The colorimetric comparison is not accurate for the shade of colour depends on the type of hair used."

4. MODE OF PERFORMING THE TEST AND READING OF RESULTS.

The test itself is carried out in the following manner:- Ten Wassermann tubes, to be used as mixing tubes, are placed in a row in a rack, and opposite them a corresponding row of narrow agglutinating tubes. By means of a

fine graduated pipette 0.4 cc. of distilled water is placed in each of the ten Wassermann tubes, and to the first tube 0.4 cc. of the patient's serum is added. The contents of the first tube are thoroughly mixed by drawing them up into the pipette several times, after which 0.4 cc. of 1 in 2 dilution of serum is withdrawn and added to the second tube, from which in turn, after thorough mixing, 0.4 cc. of the contents is withdrawn and added to the third tube. The process is repeated until the ninth tube is reached. From this 0.4 cc. is withdrawn and discarded. With a clean pipette, 0.4 cc. of the melanin solution is added to each of the ten tubes. A fresh pipette or Pasteur quill tube is now used to trasfer the contents of each mixing tube to the corresponding agglutinating tube in front of it, commencing at the tenth tube and working backwards. Thus the ultimate dilutions of the patients' serum obtained are 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024. The tenth, or control tube, contains melanin solution plus distilled water only. In the present investigation it has been found that there is little to be gained by taking the dilution to such a high figure. For all practical purposes 1/128 suffices. When the reaction is positive in this dilution it is usually very weak while positive re-

sults are not very frequently obtained in dilutions greater than 1/64. The series of agglutinating tubes thus set up should prove on examination to be clear and transparent. They are incubated at 37°C for five hours and the results read at the end of that time. Positive results are easily observed on naked eye examination and are indicated by the presence of a fine brown precipitate occurring at the bottom of the tube. A general haziness of the column must not be interpreted as being positive.

5. NATURE OF THE REACTION.

The exact nature of the reaction has still to be determined. Various suggestions have been put forward, but no satisfactory explanation has yet been given. As stated earlier in the paper, Henry (1934) has consistently favoured the view that the reaction occurs as the result of interaction between melanin pigment functioning as antigen, and a corresponding antimelanin-agglutinin developed in human sera after malarial infection. Le Bourdelles and Liégois (1929) likewise concluded that the melanin plays an active and probably antigenic part in the reaction, while Kritschewski and Demidowa (1933), experimenting with malaria in birds, came to the con-

clusion that the phenomenon is a reaction between antigen, the melanin of the retina, and antibodies, the latter being formed in the animal body by the action of the melanin of the malaria parasite. Chorine and Gillier (1933) however have suggested that the reaction is due to an increase in the serum globulins accompanied by a disequilibrium in the other soluble constituents of serum. They have shown that negative sera can be transformed to positive sera by the addition of four volumes of 1/2000 formalised distilled water, that positive sera become negative when heated at 55°C. for half an hour, and that the melanin pigment used for the test proved to be non-antigenic by animal immunisation. Greig, van Rooyen and Hendry (1934a) favour the latter view, since, by a number of experiments they found the reacting principle in positive serum to be thermolabile at the relatively low temperature of 55°C. and that melanin pigment failed to produce agglutinin response after repeated innoculation into rabbits. Consequently they consider that the phenomenon cannot be regarded as a true flocculation following interaction between antigen and antibody and suggest the term melano-precipitation is more correct than melano-flocculation. These observers, by demonstrating that dioxyphenylalanine,

the natural precursor of melanin, also gives a positive reaction, refuted the idea that the reaction might be due to some other substance or substances which happen to be present in colloidal solutions obtained from hair in the preparation of the solution used. Trenz (1932) has also come to the conclusion that the reaction is not one between melanin and its antibody. He found it positive in other protozoal infections in which no melanin is produced, and therefore attributes it to an instability of serum which occurs in protozoal diseases. Chorine and Gillier (1933) have further suggested that the reaction is due in whole or in part to an increase in the blood of such substances as lecithin, cholesterol and uric acid, but it has been shown by Fairley and Bromfield (1933) that in malaria there is a hypocholesterolaemia, and in the present investigation this has been confirmed as is demonstrated by the results shown in table A. Thus the reaction cannot be considered as due to an increase of cholesterol. The same workers on the other hand produced evidence to show that an increase of blood urea occurred in malaria, but observations made during the present investigation tend to prove that the blood urea is generally within normal limits - table B.

TABLE A.

BLOOD CHOLESTEROL IN MALARIA.

Serial Letter.	Type of Malaria.	Blood Cholesterol mgms/100 ccs.
D	B.T.	72
A	S.T.	86
V	S.T.	97
M	S.T.	107
B	S.T.	97
F	S.T.	93
C	B.T.	114
S	B.T.	73
Ba	S.T.	97

B.T. = Benign tertian.

S.T. = Sub-tertian.

Average for 9 cases = 91.8 mgms/100 ccs

Fairley and Bromfield (1933) consider the normal range of blood cholesterol for tropical patients to be 120 to 200 mgms.per cent. Thus all these cases show a hypocholesterolaemia and the average for the nine cases is well below the normal range.

These estimations were made by the method of Myers and Wardell (1918).

TABLE B.

BLOOD UREA IN MALARIA.

Serial Letter	Age	Parasite	Blood Urea mgms/100ccs.
N	40	S.T.	40
M	32	S.T.	42
W	32	B.T.	30
Mu	24	B.T.	42
Me	53	S.T.	59
J	44	B.T.	29
Ma	24	B.T.	54
C	23	B.T.	47
Mo	21	B.T.	31
S	27	S.T.	40
C	31	B.T.	27
P	31	B.T.	26

B.T. = Benign tertian.

S.T. = Sub-tertian.

Average Age = 31.8 years.
Average % Blood Urea = 38.9

Thus, taking 15 to 40 mgms. per cent as the normal range for blood urea the average figure in this series of cases comes within that range, and considering the cases individually the figures for the greater part are normal for the respective ages.

Greig, van Rooyen and Hendry (1934a) have come to the conclusion that phospholipoids are intimately bound up with the melano-precipitation reaction and have published graphs to support the view that any rise in titre of the reaction is accompanied simultaneously by a fall in the lipoid phosphorus and vice versa, at the same time proving that the reaction is not due to an increase in lecithins. Thus, while the reaction definitely cannot be due to an increase of cholesterol or lecithin in the blood it may, in some way, be associated with the fall of cholesterol and lipoid phosphorus, but actual proof of this is still lacking.

6. SIMILAR REACTION PRODUCED BY DISTILLED WATER.

While the present investigation was in progress, it was noticed in estimating blood-urea that when 0.2 cc. of serum was added to 2.0 cc. of distilled water, precipitation frequently occurred. A mental note was made of the degree of turbidity produced, and this was later found to correspond with the reaction obtained in the melano-precipitation tests. Consequently, in carrying out these tests it was decided to run a parallel series of controls containing serum and distilled water only, the melanin

solution being excluded. The result has been that under these conditions exactly similar precipitates were obtained except that the precipitate in the distilled water controls was white, while that in the melanin tubes was brownish in colour owing to the presence in them of the melanin solution. In fact, on occasions, the reaction in the water control has been positive in higher titre than in the melanin tubes; further, not infrequently where the cases of malaria had parasites in the blood the melanin tubes gave a negative result while the water controls have been quite strongly positive. Finally, throughout the investigation every case (except one) which gave a positive result in the melanin tubes did so also in the controls with distilled water. These facts are exemplified in the tables shown later in the paper.

On further reviewing what has been written on Henry's test it was discovered that previous workers, employing the original technique, had already noted that the reaction sometimes occurred with distilled water alone, and this phenomenon was called "surflocculance". Trensz (1932) considered the difference between this and the flocculation in the melanin tubes to be a matter of degree only, Henry's reagent assisting the flocculation. He infected 11 guinea-

pigs with trypanosomes (T.berberum) and found that the reaction became positive soon after the appearance of trypanosomes in the blood, and as the reaction became stronger "surflocculance" occurred in six of the control tubes. Chorine (1933) on the other hand does not agree with Trensz that the two phenomena are identical.

From the results obtained in the present investigation it seems clear that the two phenomena are one and the same and that the melanin merely adds a brown colour to the precipitate. It appears that the reagent tends if anything to hinder the reaction, but whether it does so as a result of its pH factor, chloride, or other salt content has not been determined. In this connexion it is interesting to note that in all cases where positive results have been obtained either in the melanin tubes or water controls alone, or in both, the reaction has been negative in a parallel series of normal saline controls which it was later decided to run. This suggests that the salt content of the solution in itself tends to inhibit the reaction. Thus while it is difficult to account for the true nature of the reaction, it would appear from the observations here made that the precipitate is in all probability a globulin. Furthermore, the pigment produced

in malaria has been identified as haemozoin (Wats and White 1932) which makes it still more difficult to understand why melanin should be regarded as responsible in any way for the reaction. In view of these findings no other conclusion seems possible, as pointed out by the writer in a preliminary investigation on the subject (Wiseman 1934) than that the melanin which has been employed in the test since its inception plays no real part in the reaction. On this account and quite apart from the fact that it is difficult to prepare, it could be abandoned. Finally the two terms melano-flocculation and melano-precipitation would appear to be misnomers, as they are based on misconceptions as to the real nature of the reaction.

Since the writer (Wiseman, 1934), using the improved technique, pointed out that the reaction occurred with distilled water and that the melanin merely acted as an indicator, the subject has been further investigated by Greig, van Rooyen and Hendry (1934b) who have confirmed his findings. They claim that there is some irregularity in the precipitation caused by distilled water as compared with that occurring in the melanin tubes, a fact which the investigation described in this thesis appears to con-

confirm. They maintain that while the reaction becomes weaker the higher the titre in the melanin tubes, this is not always the case in the distilled water tubes and they consider that this is probably due to two factors which are balanced against each other. In the first place there is a decreasing concentration of globulin which will diminish the quantity of the precipitate, and in the second place the increasing proportion of water which will tend to make the precipitate more complete. With such a balancing of two opposing factors it is not to be expected that in distilled water the amount of precipitate will diminish regularly in a series of dilutions. A third factor which they think should be considered is the concentration of hydroxyl ions, which will have a considerable effect in reducing the amount of protein precipitated.

7. THE CHEMISTRY OF HENRY'S REACTION.

Brief references have been made by various workers from time to time to the more intimate chemistry concerned in Henry's reaction. Thus, Chorine and Gillier (1933) stated that the reaction is governed by (1) an increase of substances which are soluble in distilled water, and (2) the molecular concentration of the blood, and it is due to a disturbance in the proportion of these two factors.

The authors had previously shown that melanin does not possess antigenic properties. They consider that during a malarial paroxysm the potassium in the blood rises sharply with the destruction of many blood cells, and a positive reaction tends to become negative. In other words the addition of an electrolyte to a slightly positive serum will render the latter negative. The excess of potassium is quickly eliminated at the end of the attack and the reaction is restored.

More recently Greig, van Rooyen and Hendry (1934b) have made a very full investigation into "The Chemistry of Malarial Serum with reference to the Factors concerned in the Melano-precipitation Test." They find that cholesterol and chloride figures lie within the normal values, and consider that there is justification for concluding that the reaction is not connected with gross changes of these substances. Though this statement is substantially correct it should be pointed out that in table A of this paper there is shown to be a hypocholesterolaemia in malarial paroxysms, a fact previously noted by De Paulo Santes (1916), Porak (1918), Crespin and Zaky (1919), Borel, Pons, Advier and Guilliam (1926) and Fairley and Bromfield (1933) while Wats and Gupta (1934) have found the blood cholesterol

of monkeys to be lowered in malaria. By means of a series of estimations of albumin and globulin, they (Greig, van Rooyen and Hendry) prove that the reaction is not due to any gross increase in the concentration of either of these protein factors. They consider that the precipitate obtained in distilled water is probably identical, as pointed out by the present writer (1934), with that obtained in the melanin tubes, and that of all the constituents of serum the precipitate is most likely to be protein in character, since on incubation with distilled water only, it occurs in the form of a fine, white, gelatinous mass. Furthermore it dissolves on the addition of one drop of 5 per cent neutral sodium chloride solution - a reaction typical of proteins of the globulin class. They consider that in view of their chemical findings, there is ample evidence available that the precipitate is protein in nature, and of all the globulin fractions which occur in serum, the fraction which is most likely to be precipitated by the addition of water is the euglobulin one.

Lloyd and Paul (1928) have already shown that the euglobulin content of serum is markedly increased in kala azar, and it is known that Henry's test is positive in that disease. The same workers (1929) prove that the eu-

globulin fraction is abnormally high in the serum of malarial patients. These facts all tend to suggest that the reaction occurs as a result of an increase of the euglobulin fraction of globulin in the blood serum, and that the precipitate itself is euglobulin. Against this view is the fact that Lloyd (1932) has shown that in syphilis the euglobulin is increased to a far greater extent than in malaria, but neither the present author - as will be gathered from the tables to be presented shortly - nor Greig, van Rooyen and Hendry in their recent work have found a positive Henry test in syphilis uncomplicated by malaria. From this it would appear that the mere increase in the euglobulin fraction is not the sole cause of the precipitation in Henry's reaction. Whether or not another factor is to be found in the close relationship between the titre of the melanin tubes and the concentration of the serum phospho-lipoids still remains to be determined.

8. GENERAL CONCLUSION.

To summarise briefly, the opinion formed as a result of observations made during this investigation associated with the findings of other workers is that the test is not antigenic in nature; the melanin per se acts only as

an indicator and does not take any real part in the reaction; the precipitation is associated with an increase of euglobulin in the serum, but there must be some other factor or factors concerned; the precipitate itself is globulin in nature and probably euglobulin.

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III.

THE CLINICAL APPLICATION AND VALUE OF HENRY'S TEST.

1. OUTLINE OF INVESTIGATION.

For the purpose of assessing the clinical value of Henry's reaction 125 sera have been tested and the cases divided into 9 classes as follows:-

(1) Cases of malaria with parasites in the blood.
(2) Cases of malaria after treatment.
(3) Cases of "old malaria".
(4) Cases of chronic or latent malaria.
(5) Healthy patients who had never been abroad and whose Wassermann reactions were negative.
(6) Patients who had never been abroad but who were suffering from untreated syphilis in one form or another, and at the time of testing had positive Wassermann reactions.
(7) Cases of kala azar.
(8) Cases of human and guinea-pig trypanosomiasis.
(9) Patients suffering from other diseases.

2. DETAILED RESULTS.

These results will now be presented in turn, various conclusions drawn, and comparison made with the results obtained by other workers.

1. Cases of Malaria with Parasites in the Blood.

Tables IA, IB and IC are concerned with cases which had malarial parasites in the blood, and which for the greater part were having rigors at the time the tests were made. In these tables 28 cases are considered. The first point to be noted is that as regards the melanin tubes alone, 8 of the cases, i.e. 28.6 per cent, gave a negative result. Previous workers, employing Henry's original technique, also found that not infrequently negative results are obtained where parasites are present in the blood. Thus, Le Bourdelles and Liégois (1929b) obtained 26% negative results, a figure which closely agrees with that obtained above, and Biasiotti (1933) 19 per cent negative results, while Corradetti (1932) has stressed the fact that if the blood is taken during fever or during digestion, the reaction may be weak or negative. On the other hand, Meerseman and Lacour (1933), in a series of 22 cases with parasites in the blood, obtained 100 per cent positive results, and Cartaná (1931) in a series of 36 similar cases, found 97 per cent positive. However, there is sufficient evidence presented above, both in the tables shown and in the references to the results of other workers to prove that negative results may be obtained with Henry's

TABLE IA.

CASES OF MALARIA WITH PARASITES IN THE BLOOD.

Serial No.	Parasite	Melanin.						Remarks.
		1/4	1/8	1/16	1/32	1/64	1/128	
1	S.T.	+	+	+	+	-	-	
2	S.T.	+	+	+	+	-	-	
3	S.T.	+	+	+	+	+	+	
4	S.T.	-	-	-	-	-	-	Been taking quinine
5	B.T.	+	+	-	-	-	-	
6	B.T.	+	-	-	-	-	-	
7	B.T.	-	-	-	-	-	-	
8	B.T.	-	-	-	-	-	-	
9	B.T.	+++	+++	++	-	-	-	
10	B.T.	+	-	-	-	-	-	
11	B.T.	+	+	-	-	-	-	W.R. +++
12	B.T.	++	-	-	-	-	-	

S.T. = Sub-tertian

B.T. = Benign tertian.

TABLE IB.

CASES OF MALARIA WITH PARASITES IN THE BLOOD.

Serial No.	Parasite	Melanin						Water Control						Remarks
		1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128	
13.	B.T.	++	++	+	–	–	–	+	+	++	+			
14.	B.T.	–	–	–	–	–	–	+	+	–	–	–	–	
15.	B.T.	–	–	–	–	–	–	–	–	–	–	–	–	
7.	B.T.	–	–	–	–	–	–	+	+	–	–	–	–	A relapse case.
16.	B.T.	++	–	–	–	–	–	++	++	++	+++	+	+	W.R. ++ Kahn +++
17.	S.T.	+	+	+	–	–	–	+	+	–	–	–	–	

B.T. = Benign tertian.

S.T. = Sub-tertian.

TABLE IC.

CASES OF MALARIA WITH PARASITES IN THE BLOOD.

Serial No.	Parasite	Melanin						Water Control						Saline Control						Remarks.
		1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128	
18	B.T.	+++	+++	+++	–	–	–	+++	+++	+++	+	+	+	–	–	–	–	–	–	
19	B.T.	+++	+++	++	+	–	–	+++	+++	++	+	+	+	–	–	–	–	–	–	
20	B.T.	–	–	–	–	–	–	+	+	+++	++	+	+	–	–	–	–	–	–	
21	B.T.	++	++	++	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
22	B.T.	+++	+++	+++	++	+	+	+	+	–	–	–	–	–	–	–	–	–	–	
23	B.T.	++	++	++	+	+	+	–	+	–	–	–	+	–	–	–	–	–	–	
24	S.T.	–	–	–	–	–	–	++	++	++	+	+	+	–	–	–	–	–	–	W.R. ++ Kahn – ve.
25	S.T.	+++	+++	+++	+	–	–	+++	+++	+++	++	+	–	–	–	–	–	–	–	
26	S.T.	++	+	+	+	–	–	+	+	+++	–	–	–	–	–	–	–	–	–	
27	S.T.	++	++	++	+	+	+	+	++	–	–	–	–	–	–	–	–	–	–	

B.T. = Benign tertian.

S.T. = Sub-tertian.

serum precipitation test, even where parasites are present in the blood. Furthermore as has already been suggested this may be due to the addition of an electrolyte in the form of potassium, the concentration of which rises in the blood during a malarial paroxysm. Actually, the occurrence of negative results where parasites are present in the blood is not a fact of great clinical importance for the demonstration of parasites in the blood is the most satisfactory method of establishing a diagnosis of malaria; it is all the proof that is required, and is a much quicker and more accurate means of arriving at a diagnosis than any serological test can be. Where the serum precipitation test would be of real value, if its specificity be sufficiently established, is in cases of suspected chronic or latent malaria where parasites cannot be demonstrated in the peripheral blood. The value of the reaction as an aid to diagnosis in this type of case will be considered presently.

The next point to be gathered from the tables presented is that frequently where negative results are obtained in the melanin tubes, positive results have been obtained in the water controls, a fact which, as previously pointed out, suggests there is some factor in the melanin

solution that tends to hinder the reaction. Only in one instance was a negative result obtained in the water control where the melanin series were positive (Case No.21). Furthermore, as a general rule, the reaction in the water controls is stronger and occurs in a higher dilution than in the melanin tubes. It will be observed, however, that there is an irregularity in the precipitation occurring in the distilled water as compared with that occurring in the melanin tubes, i.e. the reaction becomes weaker the higher the titre in the melanin tubes but this is not always so in the case of the distilled water tubes. As pointed out in an earlier part of the paper Greig, van Rooyen and Hendry (1934b) have recently commented on this and put forward a reasonable explanation. Thus, while the melanin solution tends to inhibit the reaction, to a certain extent it has at the same time a stabilising influence on the reaction. Hence, in applying the test clinically, they maintain, one cannot abandon the melanin entirely as it is not possible to base the results solely on the readings obtained with distilled water. Nevertheless, it is worthy of repetition, the melanin per se only acts as an indicator and is by no means an essential factor in the reaction. In fact, from the results obtained in

the present investigation there is no evidence that it is of any assistance in the reading of the tests. It could, without any real loss, have been excluded. This being the case it should be possible to find another solution of similar pH and salt content, which will act as a stabilising agent and yet be less troublesome and expensive to prepare than the melanin solution. Meanwhile, on the explanation given by the workers mentioned above, they claim it is quite possible for the reaction in distilled water to be completely absent in the lower dilutions and be present in the higher dilutions, thus being missed.

Further information of interest can be gleaned from column three of the results in table IC, where it will be seen that in every instance the reaction was absent in normal saline controls. The significance of this with regard to the virtue of the melanin solution has already been remarked upon. From the clinical point of view I would suggest that this is a good means of control. It will be seen from these and the ensuing tables that the reaction NEVER occurs in saline and therefore any apparent precipitation in saline would indicate a false positive.

Finally, it may be pointed out in passing that where positive Wassermann reactions have been obtained in the

above and ensuing cases it does not necessarily follow that the patient had syphilis (unless definitely stated) as Eller (1932) has shown, that the usual serological methods and technique employed in the diagnosis of syphilis are liable to give positive results in malaria, in persons not suffering from syphilis, while the reaction cannot be distinguished from the positive reactions of syphilis except by use of the Meinicke test. As will be shown below, a positive Henry reaction has never been obtained in the present investigation in syphilis uncomplicated by malaria. Furthermore, none of the cases (except one) here shown with positive Wassermann reactions received anti-syphilitic treatment, because these reactions eventually became negative without such treatment, while on clinical grounds it was quite evident that the patients had never had syphilis.

2. Cases of Malaria after treatment.

The next series of cases to be considered is that in which, with two exceptions, the patients were all "proved malarias" in that a short time previously parasites had been found in the blood. The serum precipitation test was carried out at varying intervals after treatment. These results are presented in tables IIA, IIB, and IIC. The

TABLE IIA.

CASES OF MALARIA AFTER TREATMENT.

Serial No.	Parasite	Melanin. 1/4	1/8	1/16	1/32	1/64	1/128	Interval Between Commencement of Treatment and Test.	Remarks.
10	Nil.	++	-	-	-	-	-	4 days	Formerly +ve 1/4
7	Nil.	-	-	-	-	-	-	4 days	-ve when parasites were present.
9	Nil.	++	++	++	+	-	-	6 days	Formerly +ve 1/16
28	Nil.	-	-	-	-	-	-	2 weeks	
1	Nil.	-	-	-	-	-	-	3 weeks	Formerly +ve 1/32
2	Nil.	-	-	-	-	-	-	3 weeks	Formerly +ve 1/32
3	Nil.	+	+	+	-	-	-	3 weeks	Formerly +ve 1/128
3	Nil.	-	-	-	-	-	-	3½ weeks	" " "
6	Nil.	+++	+++	++	+	-	-	3½ weeks	Formerly +ve 1/4 W.R. +++
6	Nil.	++	++	++	++	-	-	4 weeks	Kahn ++++ Climatic Bubo
29	Nil.	-	-	-	-	-	-	4½ weeks	Still taking quinine.

TABLE IIB.

CASES OF MALARIA AFTER TREATMENT.

Serial No.	Parasite	Melanin						Water control						Interval Between Commencement of Treatment and Test	Remarks
		1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128		
31	Nil.	+	+	+	+	+	+	+	+	+	+	+	−	7 days	Had W.R.+++ and Kahn ++++ but not present syphilis.
32	Nil.	−	−	−	−	−	−	+++	++	+	+++	+	−	7 days	Treated on clinical grounds. Spleen +
4	Nil.	−	−	−	−	−	−	+	+	−	−	−	−	8 days	-ve when parasites were present.
33	Nil.	−	−	−	−	−	−	+	+	++	−	−	−	10 days	Treated on clinical grounds. Spleen +
11	Nil.	−	−	−	−	−	−	−	−	−	−	−	−	7 weeks.	

TABLE IIC.

CASES OF MALARIA AFTER TREATMENT.

Serial No.	Parasite.	Melanin						Water Control						Saline Control.						Interval Between Commencement of Treatment and Test.	Remarks
		1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128		
34	Nil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 weeks	
16	Nil.	+	+	+	+	+	+	+	++	++	+++	+	+	-	-	-	-	-	-	3 weeks	Had two 5 day courses of atebrin
2	Nil.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7 weeks	
19	Nil.	++	++	++	++	+	+	+	++	++	+++	+	+	+	+	-	-	-	-	10 days	

two exceptions, numbers 32 and 33, had been treated on clinical grounds alone, i.e. they had been having attacks of fever which was malarial in type while the spleens were enlarged; though no parasites were found, no other diagnosis than malaria would account for the condition. Unfortunately the conclusions which can be drawn from this series are limited, as opportunity did not always present itself of carrying out the test before commencement of treatment. The tests were made at varying intervals of from 4 days to 7 weeks after commencement of treatment. The commencement of treatment has had to be taken rather than the completion of treatment, because the cases were treated by different methods according to the preference of the physician in charge of the respective cases. Thus, some were given a five day course of atebrin. In a few, this was followed by a further five day course of atebrin after an interval of five days. Again, some were given a ten day course of atebrin, and in some instances this was combined with quinine, and the latter administered for a number of weeks. These details have been omitted from the tables as they would have rendered the latter too complicated. By considering the commencement of treatment too, in preference to the completion of treatment, a better idea can be gathered as to the earliest moment the test becomes

negative. Thus, cases 1 and 2, which were formerly both positive (1/32), were both negative 3 weeks after commencement of treatment, and case 3 positive (1/128) before treatment was more weakly positive (1/16) 3 weeks after commencement of treatment, but completely negative 3 to 4 days later. With regard to cases 7 and 14, while these are shown as negative after 4 and 8 days respectively, it must be remembered - as will be seen by reference to the former tables - that these particular cases were negative at the outset, when parasites were present in the blood. In contrast to the cases quoted, it will be observed that case 16 was still positive both in the melanin tubes and the water controls, 3 weeks after the commencement of treatment, despite the fact that two five day courses of atebrin were administered with an interval of five days between each course. Another point of interest is observed in the instance of case number 9, where before treatment was started the reaction was positive (1/16) but a few days after commencement of treatment it became more strongly positive (1/32). This of course is quite in keeping with the observation already made that during fever the reaction may be weak. Apart from case 6 none of the cases following treatment has shown a positive reaction after 4 weeks, but with regard to case 6 it has to be borne in mind that this was not a case of "pure"

malaria but a case of malaria complicated by climatic bubo and a syphilitic infection.

With reference to tables IIB and IIC, it is seen that the reaction in distilled water persists longer than in the melanin tubes. As in former tables, where positive results were shown, the saline controls were all negative.

Thus it may be said in view of the above observations, that the effect of anti-malarial treatment on the serum precipitation test is generally to render the latter negative in three to four weeks, and that the reaction persists longer with the distilled water test than with the melanin test. As will be pointed out when the next set of tables is considered, a positive result in this investigation has never been obtained more than three months after an attack of malaria, and even then the reaction has been weak. Cherefeddin (1930) gives the period as less than three weeks, as in a series of 10 positive cases anti-malarial treatment produced a negative result in one to two weeks. Although it has not been the experience of the present author, several workers have found the reaction to persist for some considerable time after treatment; in fact Viallate and Remontet (1930) state that the reaction persists long after the disappearance of the malaria parasites and all other indications for the continuance of quinine treatment, while

Corradetti (1932) has formed the opinion that though quinine medication may render the reaction negative it remains positive in chronic malaria in spite of quinine. In view of this, together with the fact that, as pointed out in this paper, in a typical case of malaria the reaction may be negative throughout, the plea put forward by Le Bourdelles and Liégois (1929b) that the test should be employed to control the quinine treatment of malaria in the same way as the Wassermann reaction is used to regulate the salvarsan treatment of syphilis, cannot be wholeheartedly supported.

3. Cases of "Old Malaria".

The third series of cases consists of "old malarias". These might have been considered with the former series with regard to the disappearance or persistence of the reaction after treatment, but whereas the patients of the second series had all suffered from malaria either a few days or weeks previously, in this third series malaria had not been experienced for several months or years. Furthermore, the patients of this latter series were not admitted to hospital because of past malaria but for some other reason or illness. By considering them separately, more can be gathered as regards Henry's reaction under other

conditions. Twenty-eight cases in all are considered and these have been subdivided as follows:-

A. Patients who had recently returned from the tropics but had not experienced malaria for some months (9 cases).

B. Patients who had recently returned from the tropics but had not experienced malaria for some years (8 cases).

C. Patients who had returned from the tropics and been resident in the United Kingdom for three or more years (11 cases).

The results are set forth in tables IIIA, IIIB, and IIIC, which call for very little comment. In table IIIA, four cases are seen to give positive results in melanin. Number 39 gives a weak positive in melanin and a strong positive in distilled water, but this patient was suffering from pyonephrosis with daily fever and the possibility of this continued fever upsetting the blood chemistry sufficiently to account fully for the occurrence of the reaction in distilled water has to be considered. The other three cases showing positives in melanin and in water (numbers 40, 41 and 42) all had their last attack of malaria three months previously, and the serum precipitation reaction had persisted in a weak state. One case was suffering from climatic bubo, and another from ankylostomiasis, but these conditions quite definitely do not account for

TABLE IIIA.

CASES OF OLD MALARIA.

Serial No.	Time since last attack	Melanin	Water Control	Saline Control	Remarks
35	Several Months	Negative	-	-	Schistoso-miasis.
36	Several Mpnths	Negative	-	-	Climatic Bubo.
37	Several Months	Negative	Positive	-	Bacterial endocarditis with daily temp. for last 6 mths.
38	Six Months	Negative	Negative	-	Lumbago.
39	Ten Months	Positive 1/4	Positive 1/128	Negative	Pyonephrosis with daily temperature.
40	Three Months	Positive 1/8	Positive 1/16	Negative	Climatic Bubo.
41	Three Months	Positive 1/16	Positive 1/32	Negative	Old filariasis
42	Three Months	Positive 1/16	Positive 1/32	Negative	Anhylostomiasis
43	Several Months	Negative	Negative	Negative	Chronic Appendicitis

— = Not tested.

TABLE IIIB.

CASES OF OLD MALARIA.

Serial No.	Time since last attack.	Melanin	Water Control	Saline Control	Remarks.
44	Ten years	Negative	Negative	-	Fractured nose.
45	Several Years	Negative	Positive 1/8	-	Filariasis and G.C.arthritis.
46	Several Years	Negative	Negative	Negative	Tropical ulcers.
47	Two Years	Negative	Negative	Negative	Aortic Incompetence W.R.++
48	Three Years	Negative	Negative	Negative	
49	Seven Years	Negative	Negative	Negative	
50	Nine Years	Negative	Negative	Negative	Spleen
51	Fifteen Years	Negative	Positive 1/4	Negative	

- = Not tested.

TABLE IIIC.

CASES OF OLD MALARIA.

Serial No.	Time returned from Tropics	Melanin	Water Control	Saline Control	Remarks.
52	Several Years	Negative	-	-	Spleen. (SPRUE)
53	More than 3 years	Negative	-	-	
54	Three & half years	Negative	-	-	Chronic Cholecystitis
55	Three Years	Negative	Negative	-	Colitis.
56	Three Years	Negative	Negative	-	
57	Four & half years	Negative	Positive	-	Spleen.
58	Several Years	Negative	Negative	-	
59	Four Years	Positive 1/64	Positive 1/64		Agranulocytosis.
60	More than four years.	Negative	Negative	Negative	Sub-acute Bact. endocarditis with daily T°
61	Fifteen Years	Negative	Negative	Negative	B. Coli bacilluria with daily T°
62	Fifteen Years	Negative	Negative	Negative	

- = Not tested.

positive reactions, other cases of similar nature proving negative. The remaining case, number 41, was an old case of filariasis; no evidence of filarial infection could be found at the time of examination, and there is no reason to consider this condition as playing any part in the reaction, especially when it is considered that in table IIIB there is shown another case with definite filariasis, and although a weak positive result (1/8) was obtained in water, the reaction was negative in melanin.

The main point to be gathered from this table is, that weak positive results have been obtained in three cases, each three months after the last attack of malaria. None of these was considered to be a case of latent malaria, or to be in need of further anti-malarial treatment, facts which indicate very clearly that where a weak positive result is obtained the result must be interpreted with due regard to the history and clinical findings.

Of the remaining nineteen cases shown in Tables IIIB and IIIC, only one positive result is shown. That occurred in a patient who had not been in the tropics for four years. She was suffering from a granulocytic angina with a daily temperature varying between 101°F. and 104°F, and the upset of blood chemistry consequent on this continued fever must surely account for the positive result. The reaction occurr-

ed very strongly in the individual tubes up to 1/64 and cannot have been due to any attack of malaria occurring more than four years previously. Thus, while the last attack of malaria in these cases occurred from between two and more than fifteen years previously, not a single positive result has been obtained. Finally, the saline controls again have always proved negative.

The other diseases mentioned in the tables will be considered in another part of the discussion.

4. Cases of Chronic or Latent Malaria.

The next set of tables concerns cases of so-called "chronic malaria", and it is when these are considered that the real clinical value of Henry's reaction can be estimated. It was as an aid to the diagnosis of cases such as these that it was at one time hoped the reaction would prove to be of considerable value; a hope which numerous workers still consider has been fully justified. In this investigation 14 cases of "chronic malaria" have been tested and the results are set forth in tables IVA and IVB.

By the term "chronic malaria" is meant the type of case where repeated attacks of fever are complained of, the attacks corresponding either wholly or in greater part with what the patient has experienced formerly when known

TABLE IVA.

CASES OF CHRONIC OR LATENT MALARIA.

Serial No.	Parasite.	Melanin	Remarks.
7	Nil.	Negative	See text.
63	Nil.	Positive 1/3x	Typical malaria attack 2 months ago, lasting 9 days Spleen ++
64	Nil	Negative	Returned from tropics 2 months; repeated attacks of fever. Spleen +
65	Nil.	Positive 1/4	Typical attack (5 days) 1 weeks previously. Spleen +
66	Nil.	Positive 1/6	Typical attack 3 weeks ago; took atebrin and quinine. Spleen +
67	Nil.	Negative	Returned from India 2 years ago; repeated attacks of fever. Spleen +
68	Nil.	Positive 1/4	Recently returned from East; typical attack one week ago.

TABLE IVB.

CASES OF CHRONIC OR LATENT MALARIA.

Serial No.	Parasite.	Melanin	Water Control	Saline Control	Remarks
14	Nil.	Negative	Negative	-	See text
32	Nil.	Negative	Positive 1/16	-	See text
69	Nil.	Negative	Negative	Negative	Typical attacks of malaria since returning to England 2 mths. ago; been taking quinine and phasmoquine.
70	Nil.	Negative	Negative	Negative	Last proved attack 4 mths.ago; apparently had typical attack day before admission with T = 100.6°
71	Nil.	Positive 1/32	Positive 1/8	Negative	Typical attacks for last 20 days; Spleen +
72	Nil.	Positive 1/4	Positive 1/64	Negative	Old filariasis: c/ attacks fever; spleen -ve; been taking quinine.
73	Nil.	Negative	Negative	Negative	Attack 3-4 days before admission. Spleen +

- = Not tested.

to be definitely suffering from malaria. Here the history must be carefully enquired into for it is found that about 99 per cent of patients who have been abroad are inclined to designate any slight rise of temperature associated with shivering as "fever", by which they mean malaria. In the experience of the author, however, very commonly the condition is one of influenza or not infrequently that of bacillus coli infection of the urinary tract. In the cases here reviewed however the attacks described appeared to be typical of malaria while many of them were associated with enlargement of the spleen, so that the clinical evidence was sufficient to justify treatment by anti-malarial measures. The results shown are of great interest and are of vital importance in estimating the clinical value of Henry's reaction.

The type of case where such a reaction would be of extreme value is that where the patient complains of repeated attacks of what appears to be malaria but at the time of examination is free from fever while parasites are not detectable in the blood. In actual practice case number 7 illustrates what is found. The patient, W.C.B., aet 26 years, formerly a soldier, came to the hospital complaining of repeated attacks of "fever". He had spent four and a half years in India but had resided in England

since November 1933. He first contracted malaria in 1930, when benign tertian parasites were found in the blood. About three months later he had another attack, and on this occasion malignant tertian parasites were found. Ever since then he had experienced attacks from time to time, and in each instance treatment had been by means of quinine or plasmoquine. Since arriving in England he had experienced about five attacks in all, each attack being characterised by rigor, headache, sweating,and left subcostal pain. He was admitted to hospital on 18:6:34 and stated that the previous day he had had an attack of the nature described. Clinically there was found to be slight enlargement of liver and spleen, but blood examination then and again two days later failed to reveal malarial parasites. The serum precipitation test performed on admission was negative (table IVA). During routine investigation the faeces were found to contain segments and ova of Taenia saginata and cysts of Entamoeba histolytica. The tapeworm condition was therefore treated on the usual lines and following this it was decided to give treatment for the amoebic infection. On the evening of 30:6:34 he was given one grain of emetine bismuth iodide and before the second dose could be given the following evening, he had a true malarial rigor with temperature of 103°F., and benign tertian

parasites were found in the blood, but the serum precipitation test remained negative (table IA). The emetine bismuth iodide treatment was discontinued and treatment of the malaria with atebrin instituted. Two days later the patient had another rigor, somewhat milder in nature, parasites were still present in the blood and the serum precipitation test was still negative (table IIA). Here, therefore, we have a case in which Henry's reaction failed to reveal latent or chronic malaria, in which, in other words, the test was a complete failure.

Case number 14 was even more instructive. The patient, Mrs. M.C., aet 34 years, had recently arrived in England from Nigeria, where she had resided since 1925. She first contracted malaria in 1925, had had several attacks since, and on two occasions was treated in hospital. She visited the out-patient department of the hospital at 11 a.m. on 8:8:34 with a history of alternate day fever, malarial in type, of two weeks' duration; the last attack had occurred two days previously. On examination, though the patient did not look very well, there was no elevation of temperature and no enlargement of the spleen. Blood slides were taken, blood withdrawn for the serum precipitation test, and the patient asked to report again at 3 p.m. of the same day for the result of the blood slide examination. The

blood slides failed to reveal parasites and the specimen of blood was later found to give a negative Henry reaction (table IVB). When the patient reported at 3 p.m. as directed, the writer was discussing the situation with her and advising her what procedure to adopt - in fact a prescription had already been written out as one felt justified on the history in using anti-malarial measures - when she suddenly began to shiver violently. Her temperature was found to be 101.4°F. and fresh blood slides showed benign tertian parasites, but a further sample of blood still gave a negative serum precipitation reaction (table LB). Here then is another case in which Henry's test was a complete failure, even though the patient was within a few hours of experiencing a rigor.

In estimating the clinical value of Henry's reaction with regard to cases of chronic or latent malaria the investigator is immediately confronted with a difficulty. Thus a case may be considered on clinical grounds to be one of chronic malaria but there can be no actual proof of this unless parasites are found in the blood, when, of course, Henry's test as a means of diagnosis would immediately become unnecessary. The two cases described in detail above are sufficient to show that this state of affairs still confronts the investigator, as Henry's re-

action at the moment does not warrant complete faith being placed in it. This is further supported by the fact that even where a positive result is obtained, as in case number 59 (table IIIC), it is just possible that any long continued fever MAY give a positive Henry reaction. Thus it would appear that where the history of a case suggests chronic or latent malaria, parasites being absent from the blood, and the serum precipitation test is positive, the procedure to adopt is to treat the patient with anti-malarial measures, and if the reaction becomes negative then one can say that the diagnosis of malaria was correct. Unfortunately, opportunity did not present itself to allow of this being done in all the cases shown in tables IVA and IVB but in this connexion attention might profitably be drawn to case number 32. In this, the clinical evidence suggested chronic malaria. The serum precipitation test was negative in melanin but positive (1/16) in water (table IVB). Treatment with atebrin was instituted and seven days later the test, although remaining negative with melanin, gave a positive reaction (1/64) with water (table IIB) - another anomalous feature of Henry's reaction.

Of the remaining 11 cases shown, all were regarded on clinical grounds as requiring anti-malarial treatment and yet only six of these gave positive reactions, the highest

occurring in a titre of 1/32.

Thus in these cases - the type in which a test such as that advocated by Henry would have been of great value - the test has proved of little or no real aid to diagnosis.

5. and 6. Cases with Positive and Negative Wassermann Reactions.

In order to complete the investigation as to the clinical value of Henry's reaction the test was carried out under other conditions which will now be described. A series of normal, healthy, people who had never been abroad and whose Wassermann reactions were negative, were investigated. Then a series of people who had never been abroad, but whose Wassermann reactions were positive at the time of testing (single, double and triple plus), and who had not had anti-syphilitic treatment. These results are shown in table V and from these it can be affirmed that —

(a) the reaction does not occur in normal serum, even with distilled water. This observation is of importance in view of the statement put forward recently by Greig, Hendry and van Rooyen (1934b). Since the occurrence of the reaction with distilled water in the sera of malarial patients was first pointed out by the author, these workers have conducted further investigations, and maintain that

TABLE V.

CASES WITH POSITIVE AND NEGATIVE WASSERMAN REACTIONS.

Type of Case	Serial Nos.	No. of cases Tested	Results.
Healthy individual. Never been abroad. Wasserman reaction -ve.	H_1 to H_{17} inclusive.	17	Only one case gave a positive result in melanin and in that case haemolysis of the serum had occurred, therefore it has to be regarded as a false positive. In another case the reaction occurred in distilled water in 1/32 and 1/28 but not in the other titres. All saline controls were negative.
Wasserman reaction positive. No anticyphilitic treatment. No residence abroad.	S1 to S_{12}	12	All negative in melanin, in water and in saline.

the melanin solution used in the test prevents the precipitation which occurs in normal serum, and permits the precipitation only of the excess which occurs in malaria and kala azar;

(b) the reaction does not occur either with melanin or with water, in syphilis uncomplicated by malaria. This conclusion is confirmed by the above mentioned workers, and is important from the point of view of the opinion that euglobulin is in large part responsible for the reaction, because euglobulin occurs in far greater excess in syphilis than it does in either malaria or kala azar. Meerseman and Lacour (1933) similarly, found the reaction negative in 10 out of 11 syphilitics while Biasiotti (1933), Tzeclnowitza, Gorchowa and Moldavaskaja-Kritschewskaja (1932) have also found it to be negative in this disease. On the other hand, Farjot (1933) found it to be definitely positive in syphilitics who had never had malaria; in four cases with feeble Wassermann reactions the test was negative, but in three with strongly positive Wassermann reactions the test was positive.

7. A Case of Kala Azar.

Bearing in mind the marked increase of euglobulin which occurs in kala azar, an opportunity of testing the reaction in this disease was welcomed, and a strongly positive result

was obtained, as shown in table VI. The patient was a native from India. He ran an irregular temperature which was associated with a leucopaenia, enlargement of the liver, and enlargement of the spleen. No malarial parasites could be found in the blood although numerous examinations were made. Despite the fact that both hepatic and splenic punctures failed to reveal the presence of Leishman-Donovan bodies, the clinical evidence was so strongly in favour of kala azar, that intravenous injections of neostibosan were administered and improvement resulted. The occurrence of Henry's reaction in kala a zar has been noted by numerous workers including Trensz (1932), Sergent (1933), Voigtlander (1934), Greig, Hendry and van Rooyen (1934b), and is generally accepted; in fact Voigtlander (1934) in performing the test for malaria, employs a formalin control, which excludes cases of kala azar by giving a positive formol-gel reaction in that disease, and this is a procedure which should be adopted when Henry's reaction is used in areas where kala azar is prevalent.

8. Cases of Human and Guinea-pig Trypanosomiasis.

As stated earlier in the paper, Trensz (1932) has obtained positive results both in human and guinea-pig trypanosomiasis. Sergent (1933) has also obtained positive re-

TABLE VI.

CASE OF KALA AZAR.

Serial No.	Type of Case.	Serum-Precipitation Reaction.					
		1/4	1/8	1/16	1/32	1/64	1/128
K1.	Clinical kala-azar. (see text).	+++	+++	+++	+++	+++	-

sults in trypanosomiasis, but on the other hand Meerseman and Lacour (1933) emphatically state that Henry's reaction is specific for malaria and does not give positive results in other diseases, not even in trypanosomiasis for in two cases of sleeping sickness and in one guinea pig infected with trypanosomiasis negative results were obtained. In the present investigation, employing the improved technique, one case of human trypanosomiasis and two cases of guinea-pig trypanosomiasis have been tested, and from the results shown in table VII it can be stated that Henry's reaction is positive in that disease.

9. Cases of Other Diseases.

By reference to tables IIIA, IIIB and IIIC it will be seen that negative results have been obtained in schistomiasis, climatic bubo, subacute bacterial endocarditis (2 cases), lumbago, chronic appendicitis, injury, tropical ulcer, aortic incompetence due to syphilis, spleen (3 cases), chronic cholecystitis, lymphadenoma and bacillus coli infection of the urinary tract. A series of cases which includes tropical and non-tropical conditions, some of which were running temperatures of considerable degree. In a case of blackwater fever with rigor and temperature of 105°F. negative results were obtained both with melanin and distilled water, and a case of ulcerative colitis also proved negative. One case of agranulocytosis will be seen from the tables to give a strong positive reaction; the

TABLE VII.

CASES OF HUMAN AND GUINEA PIG TRYPANOSOMIASIS.

Serial No.	Type of Case.	Melanin						Water Control						Saline Control					
		1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128	1/4	1/8	1/16	1/32	1/64	1/128
H.T.1	Human trypanosomiasis	+	++	+	+	+	+	+	++	+	−	+++	−	−	−	−	−	−	−
G.T.1	Guinea-pig trypanosomiasis (T. Brucci).	+	+	−	−	−	−	+++	+++	+++	+++	++	+	−	−	−	−	−	−
G.T.2	Guinea-pig trypanosomiasis (T. Brucci).	+	+	+	+	+	−	+++	+++	+++	−	−	−	−	−	−	−	−	−

patient had been back from the tropics for four years and this must be accepted as a non-specific positive result. Tzechnowitza, Gorchowa and Moldavaskaja-Kritschewskaja (1932) have also found the reaction to be negative in a large series of non-malarial cases including syphilis, tuberculosis, carcinoma, typhoid, erysipelas, dermal leishmaniasis, as well as in septic infections. These workers, however, found the reaction to be definitely positive in typhus fever. Owing to the extreme rarity of typhus in this country opportunity has not presented itself to confirm this observation in the present investigation, but it should be pointed out that the occurrence of Henry's reaction in typhus appears to be definitely established. In addition to the above workers , Tzechnowitza, Gorchowa and Moldavaskaja-Kritschewskaja and Fridman (1933) investigated 100 cases of typhus, and found that in 95 of these, Henry's reaction became positive during the course of the fever, following closely the curve of the Weil-Felix reaction, while similar results were obtained by Karout and Moldavaskaja-Kritschevskaja (1933) in six rabbits innoculated with typhus virus, and in this connection Gerbinis (1934) has pointed out an interesting fact, viz., that although Henry's reaction is positive in typhus and follows the curve of the Weil-Felix reaction, yet the Weil-Felix reaction is not positive in malaria. Thus, we have here a means of differentiating

the two diseases where Henry's reaction is positive and typhus fever has to be eliminated.

General Discussion.

In brief, then, this investigation has shown that Henry's reaction is frequently negative where malarial parasites are present in the blood; it not infrequently fails to reveal latent malaria; it may persist where there is no clinical indication for further anti-malarial treatment; it is positive in kala azar, and human and guinea-pig trypanosomiasis; it is negative in normal serum, syphilis, blackwater fever and numerous other diseases tropical and non-tropical, and a strongly positive result has been obtained in a case of agranulocytic angina. In view of these findings, it is confidently asserted that Henry's reaction in its present state is of little clinical value. Nevertheless, a big step has been made in the right direction in the search for a test to detect latent malaria, and no doubt as further work is done on the subject, out of Henry's reaction will be evolved a test of considerable value. Further work at the moment would appear to lie in the province of the bio-chemist. These conclusions have been arrived at despite the assertions of Voigtlander (1934) who considers that "Henry's test is of considerable value in the diagnosis

of latent malaria, and fills a lacuna, which hitherto existed in the recognition of malarial infection", and Moldavaskaja-Kritschewskaja, Gritzay and Uman (1933), who consider that Henry's reaction is pathognomonic of malaria and can be employed as a useful diagnostic measure long before clinical signs appear. The results obtained in the present investigation find support to some extent in the work of Sergent (1933), who himself considers the reaction to be of comparatively little value.

The conflicting results obtained by different workers add further weight to the conclusions arrived at by the present author. The differing results can only be due to a lack of standardisation of the melanin solution as regards its pH and salt content, as very slight alterations of either of these factors are known to affect the reaction. Henry himself laid considerable stress on the need for keeping the melanin solution just alkaline. A further source of error is found in the fact that very occasionally the melanin fails to keep wholly in solution. If this is not noticed at the moment of carrying out the test, then when readings are taken, false positives may be recorded, i.e. there appears to be the usual precipitation but on closer examination this is found to consist of fine particles of melanin.

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The main conclusions arrived at as a result of this investigation may be summarized as follows:-

(1) Henry's reaction does not occur as the result of interaction between antigen and antibodies.

(2) The melanin employed acts merely as an indicator, and even as such, is of little value.

(3) The reaction takes the form of a precipitation, not a flocculation.

(4) The same precipitation occurs in distilled water without the melanin solution.

(5) The precipitation occurs irregularly in distilled water, and the melanin solution by virtue of its pH factor and salt content has a stabilising influence.

(6) The precipitate is in all probability euglobulin.

(7) The terms melano-flocculation and melano-precipitation are misnomers, and it is better to use the title serum precipitation, or Henry's reaction.

(8) Very careful preparation and standardisation of the solution used is essential in order that results by different workers may be compared.

(9) As regards the clinical value:-

(a) Henry's reaction is frequently negative where malarial parasites are present in the blood.
(b) the reaction not infrequently fails to reveal latent malaria
(c) it may persist where there is no clinical indication for further anti-malarial treatment.
(d) it is negative in normal serum and in syphilis
(e) it is negative in blackwater fever and numerous other diseases, tropical and

non-tropical.
(f) it is positive in kala-azar and in trypanosomiasis.

All things considered, Henry's reaction is of little clinical value.

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