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*J Clin Invest.* 1929;7(1):27-43. <https://doi.org/10.1172/JCI100219>.

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# OBSERVATIONS ON PAROXYSMAL HEMOGLOBINURIA<sup>1</sup>

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(Received for publication October 23, 1928)

For the discovery of the reaction which reproduces in vitro what appears to be the essential feature of the mechanism of this disease we are indebted to Donath and Landsteiner (1). They showed that the serum of these patients contains an auto-hemolysin which unites with the patient's own red blood cells or with those of any other individual only at a low temperature; that when such a mixture is warmed to 37°C. hemolysis occurs if complement be present. Complement is essential for the completion of the reaction. Rosenbach (2) had previously shown that all the phenomena of a typical paroxysm of the disease may be produced by immersion of the hands or feet of the patient in ice water.

During the past nine years I have had five of these patients under observation and this paper summarizes some of the serological, clinical and therapeutic results.

This auto-hemolysin has certain striking peculiarities. At body temperature it will not unite with the red blood cells. The highest temperatures at which union could be detected with the auto-hemolysins from three of the patients I have studied were respectively 12°C., 12°C. and 10°C. (protocol 1). Temperatures down to 4°C. or 5°C., however, gave more complete union, shown by greater hemolysis when the mixture was subsequently warmed. In two of these patients hemolysis was greater if the red cells from the patient were used rather than those from another individual of the same blood group (protocol 2). In all five of these patients it has been possible to confirm the observation of Yorke and Macfie (3) that union is more effective

<sup>1</sup> Presented in abstract form at the meeting of the Association of American Physicians, May 2, 1928.

and hemolysis greater if the chilling is limited to five or ten minutes rather than the more usual period of thirty minutes (protocol 3). This is particularly interesting in view of what occurs when a patient spontaneously has a paroxysm. The actual time during which the

# PROTOCOL 1

December 7, 1922. Patient L. O. Blood drawn from arm vein; one portion allowed to clot at room temperature; serum separated 30 minutes after venepuncture; serum in ice box over night. A second portion was taken in citrate and used for the preparation of the red cell suspension. Control serum and red cell suspension prepared in the same way from a normal adult belonging to the same blood group.

Tube	Patient's serum unheated	Patient's red blood cells 5 per cent	Control serum unheated (Group A)	Control red blood cells 5 per cent (Group A)	Complement 1:10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
	cc.	cc.	cc.	cc.	cc.	cc.		
1	0.25	0.1			0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	++
2	0.25			0.1	0.1	0.05		++
3	0.25	0.1			0.1	0.05	4°C. 10 minutes, 37°C. 2 hours	+(+)
4	0.25			0.1	0.1	0.05		++
5	0.25	0.1			0.1	0.05	8°C. 10 minutes, 37°C. 2 hours	+(+)
6	0.25			0.1	0.1	0.05		+(+)
7	0.25	0.1			0.1	0.05	12°C. 10 minutes, 37°C. 2 hours	+
8	0.25			0.1	0.1	0.05		+
9	0.25	0.1			0.1	0.05	16°C. 10 minutes, 37°C. 2 hours	0
10	0.25			0.1	0.1	0.05		0
11			0.25	0.1	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
12		0.1	0.25		0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
13	0.25	0.1			0.1	0.05	Not chilled, 37°C. 2 hours	0
14			0.25	0.1	0.1	0.05	Not chilled, 37°C. 2 hours	0
15				0.1	0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0
16		0.1			0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0

blood in superficial capillaries is exposed to lowered temperatures is obviously short. It soon passes back to the higher temperatures of the interior of the body.

These auto-hemolysins also have in some instances a surprising thermolability. In one case 45°C. for thirty minutes destroyed the auto-hemolysin so that the addition of fresh complement would not reacti-

vate it; in another case it was destroyed at 47.5°C. The extreme lability of this strange hemolysin is further shown by the fact that sometimes after one to three days in the ice-box the serum will no longer cause lysis of the red cells. The hemolysin has disappeared (protocol 6). It has been shown repeatedly that the serum of par-

# PROTOCOL 2

January 2, 1925. Patient F. V. Blood drawn from arm vein; one portion was allowed to clot at room temperature; serum separated as soon as clot had formed and test set up at once; a second portion was citrated and used for the preparation of the red cell suspension. Control serum and red cell suspension obtained from a normal adult belonging to the same blood group as the patient.

Tube	Patient's serum unheated	Patient's red blood cells 5 per cent	Control serum unheated (Group O)	Control red blood cells 5 per cent (Group O)	Complement 1:10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
	cc.	cc.	cc.	cc.	cc.	cc.		
1	0.3	0.1		.	0.1		0°C. 10 minutes, 37°C. 2 hours	++
2	0.25	0.1			0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	+ (+)
3	0.2	0.1			0.1	0.1	0°C. 10 minutes, 37°C. 2 hours	+
4	0.1	0.1			0.1	0.2	0°C. 10 minutes, 37°C. 2 hours	0
5	0.3			0.1	0.1		0°C. 10 minutes, 37°C. 2 hours	+
6	0.25			0.1	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
7	0.2			0.1	0.1	0.1	0°C. 10 minutes, 37°C. 2 hours	0
8	0.1			0.1	0.1	0.2	0°C. 10 minutes, 37°C. 2 hours	0
9		0.1	0.25		0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
10			0.25	0.1	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
11		0.1			0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0
12				0.1	0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0
13	0.3	0.1			0.1		Not chilled, 37°C. 2 hours	0
14	0.25	0.1			0.1	0.05	Not chilled, 37°C. 2 hours	0

oxysmal hemoglobinurics causes lysis not only of the patient's own red cells but also of the red cells of any other individual. I have made several attempts to separate by absorption experiments the auto-hemolysin from the iso-hemolysin (protocol 4). But in no case was one removed without also removing the other; hence they are probably identical.

Whether it is necessary to have complement present at the low temp-

erature is a question which has been answered in different ways by different observers (4, 5). So far as my observations go they indicate that complement may be added after the chilled serum and cells have been warmed to 37.5° and still have hemolysis occur, but that if alexin be present in the mixture at the low temperature, more complete hemolysis occurs (protocol 5).

#### PROTOCOL 3

November 29, 1921. Patient L. O. Blood drawn from arm vein; one portion allowed to clot at room temperature; serum separated as soon as coagulation was complete and test set up immediately; a second portion was citrated and used for the preparation of the red cell suspension. Control serum and red cell suspension obtained in the same way from a normal adult of the same blood group.

Tube	Patient's serum unheated	Patient's red blood cells 5 per cent	Control serum unheated (Group O)	Control red blood cells 5 per cent (Group O)	Complement 1:10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
	cc.	cc.	cc.	cc.	cc.	cc.		
1	0.25	0.1			0.1	0.05	0°C. 5 minutes, 37°C. 2 hours	+++
2	0.25	0.1			0.1	0.05	0°C. 7 minutes, 37°C. 2 hours	+++
3	0.25	0.1			0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	+++
4	0.25	0.1			0.1	0.05	0°C. 15 minutes, 37°C. 2 hours	++
5	0.25	0.1			0.1	0.05	0°C. 20 minutes, 37°C. 2 hours	++
6	0.25	0.1			0.1	0.05	0°C. 30 minutes, 37°C. 2 hours	+(+)
7		0.1	0.25		0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
8			0.25	0.1	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
9				0.1	0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0
10		0.1			0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0
11	0.25			0.1	0.1	0.05	0°C. 30 minutes, 37°C. 2 hours	++(+)
12	0.25	0.1			0.1	0.05	Not chilled, 37°C. 2 hours	0
13	0.25			0.1	0.1	0.05	Not chilled, 37°C. 2 hours	0

Another question upon which discordant results have been reported (6, 7) is the possible effect of CO<sub>2</sub> either as a substitute for chilling or as an auxilliary factor in bringing about union of the hemolysin and the red cells. The experiments I have done with CO<sub>2</sub> were entirely negative (8). No effect upon union nor upon hemolysis was observed.

That there is probably some undefined factor in the mechanism of the disease appears to be indicated by observations on two of our

# PROTOCOL 4

December 5, 1921. Patient L. O. Blood drawn from arm vein; one portion allowed to clot at room temperature; serum separated as soon as coagulation was complete and the experiment set up immediately; a second portion was citrated and used for the preparation of the red cell suspension. Control serum and red cell suspension obtained in the same way from a normal adult of the same blood group.

2.0 cc. of the patient's serum was mixed with 2.0 cc. of washed and packed red blood cells of the patient and immersed in melting ice for 15 minutes. The mixture was then centrifuged cold and the supernatant serum quickly pipetted off. This is "Patient's serum 'auto'-absorbed."

The same absorption of the patient's serum in the cold was carried out with an equal volume of washed and packed red blood cells from a normal individual of the same blood group. This is "Patient's serum 'iso'-absorbed."

Tube	Patient's serum "auto"-absorbed untreated	Patient's serum "iso"-absorbed untreated	Patient's serum untreated	Patient's red blood cells 5 per cent	Control red blood cells 5 per cent (Group A)	Control serum untreated (Group A)	Complement 1:10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.		
1	0.25			0.1			0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
2	0.25				0.1		0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
3		0.25		0.1			0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
4		0.25			0.1		0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
5			0.25	0.1			0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	++++
6			0.25		0.1		0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	++++
7				0.1			0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0
8					0.1		0.1	0.3	0°C. 10 minutes, 37°C. 2 hours	0
9				0.1	0.25	0.1	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
10					0.1	0.25	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
11	0.25			0.1			0.1	0.05	Not chilled, 37°C. 2 hours	0
12	0.25				0.1		0.1	0.05	Not chilled, 37°C. 2 hours	0
13		0.25		0.1			0.1	0.05	Not chilled, 37°C. 2 hours	0
14		0.25			0.1		0.1	0.05	Not chilled, 37°C. 2 hours	0

Titration of the Wasserman reaction on the "auto"-absorbed and the untreated serum used in the above experiment gave the following results:

	0.02 cc.	0.01 cc.	0.006 cc.	0.003 cc.	0.001 cc.	0.0005 cc.
<i>Untreated serum:</i>						
Alcoholic antigen.....	++++	++++	++++	++++	+	0
Cholesterin antigen.....	++++	++++	++++	++++	+	0
<i>"Auto"-absorbed serum, not inactivated:</i>						
Alcoholic antigen.....	++++	++++	++++	++++	+	0
Cholesterin antigen.....	++++	++++	++++	++++	++	±
<i>"Auto"-absorbed serum, inactivated:</i>						
Alcoholic antigen.....	++++	++++	++++	++++	++	0
Cholesterin antigen.....	++++	++++	++++	++++	+	0

patients. One was a boy of 7 and the other a boy of 8. One had an extreme susceptibility to spontaneous attacks and a low titer of hemolysin in his serum and plasma; the other had paroxysms only after relatively long exposure to low temperatures and a high titer of hemolysin in his serum and plasma. There seems to be no explanation for such an inverse relationship between the titer of hemolysin and the susceptibility to attacks unless one postulates some factor as yet undemonstrated. The fact that the boy with a low titer of hemolysin and high susceptibility to attacks was subject to very marked vasomotor disturbances while the other boy had none that were apparent,

#### PROTOCOL 5

November 11, 1920. Patient J. B. Serums, red cell suspensions and complement prepared in the usual way. One portion of the patient's serum was inactivated at 55°C. for 30 minutes.

Tube	Patient's serum un-heated	Patient's serum in-activated	Patient's red blood cells 5 per cent	Complement 1:10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
1	cc. 0.25		cc. 0.1	cc. 0.1	cc. 0.05	0°C. 30 minutes, 37°C. 2 hours	+++
2		0.25	0.1		0.05	0°C. 30 minutes, 37°C. 2 hours	0
3		0.25	0.1	0.1	0.05	0°C. 30 minutes, 37°C. 2 hours	++
4		0.25	0.1		0.05	0°C. 30 minutes. Tube warmed to 37°C. then 0.1 cc. complement added, Kept at 37°C. 2 hours	+

All the usual controls were negative

would suggest that a vasomotor mechanism may have something to do with the hemolysis.

From the etiological and from the therapeutic point of view the most important aspect of this disease is its relation to syphilis. Many of the reported cases have been in congenital syphilitics. The Wasserman reaction is positive in nearly every case and in many of the adult cases late manifestations of acquired syphilis have been present (9, 10). It was of interest therefore to attempt the separation of the auto-hemolysin from the Wassermann reacting substance. This has

# PROTOCOL 6

January 27, 1925. Patient G. C. Blood drawn from arm vein, divided into two portions; serum and red cell suspension prepared in the usual way. Test set up immediately.

Tube	Patient's serum un-heated	Patient's red blood cells 5 per cent	Complement 1:10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
	cc.	cc.	cc.	cc.		
1	0.3	0.1	0.1		0°C. 10 minutes, 37°C. 2 hours	++++
2	0.25	0.1	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	++++
3	0.2	0.1	0.1	0.1	0°C. 10 minutes, 37°C. 2 hours	++++
4	0.15	0.1	0.1	0.15	0°C. 10 minutes, 37°C. 2 hours	+++
5	0.10	0.1	0.1	0.2	0°C. 10 minutes, 37°C. 2 hours	+++
6	0.05	0.1	0.1	0.25	0°C. 10 minutes, 37°C. 2 hours	+
7	0.01	0.1	0.1	0.29	0°C. 10 minutes, 37°C. 2 hours	±

The usual controls were negative

## *Titration of the Wassermann reaction on the same serum*

	0.1 cc.	0.02 cc.	0.01 cc.	0.006 cc.	0.003 cc.	0.001 cc.
Alcoholic antigen.....	++++	++++	++++	++++	++++	++++
Cholesterin antigen.....	++++	++++	++++	++++	+++	++

The serum was then left in the ice box for 3 days. Repetition of the above titrations: January 30, 1925.

Tube	Patient's serum un-heated	Patient's red blood cells 5 per cent	Complement 1:10 (guinea pig)	NaCl 0.85 per cent	Kept at	Hemolysis
	cc.	cc.	cc.	cc.		
1	0.3	0.1	0.1		0°C. 10 minutes, 37°C. 2 hours	0
2	0.25	0.1	0.1	0.05	0°C. 10 minutes, 37°C. 2 hours	0
3	0.2	0.1	0.1	0.1	0°C. 10 minutes, 37°C. 2 hours	0
4	0.15	0.1	0.1	0.15	0°C. 10 minutes, 37°C. 2 hours	0
5	0.10	0.1	0.1	0.2	0°C. 10 minutes, 37°C. 2 hours	0
6	0.05	0.1	0.1	0.25	0°C. 10 minutes, 37°C. 2 hours	0
7	0.01	0.1	0.1	0.29	0°C. 10 minutes, 37°C. 2 hours	0

The usual controls were negative

## *Titration of the Wassermann reaction on the same serum*

	0.1 cc.	0.02 cc.	0.01 cc.	0.006 cc.	0.003 cc.	0.001 cc.
Alcoholic antigen.....	++++	++++	++++	++++	+++	++
Cholesterin antigen.....	++++	++++	++++	++++	+++	+



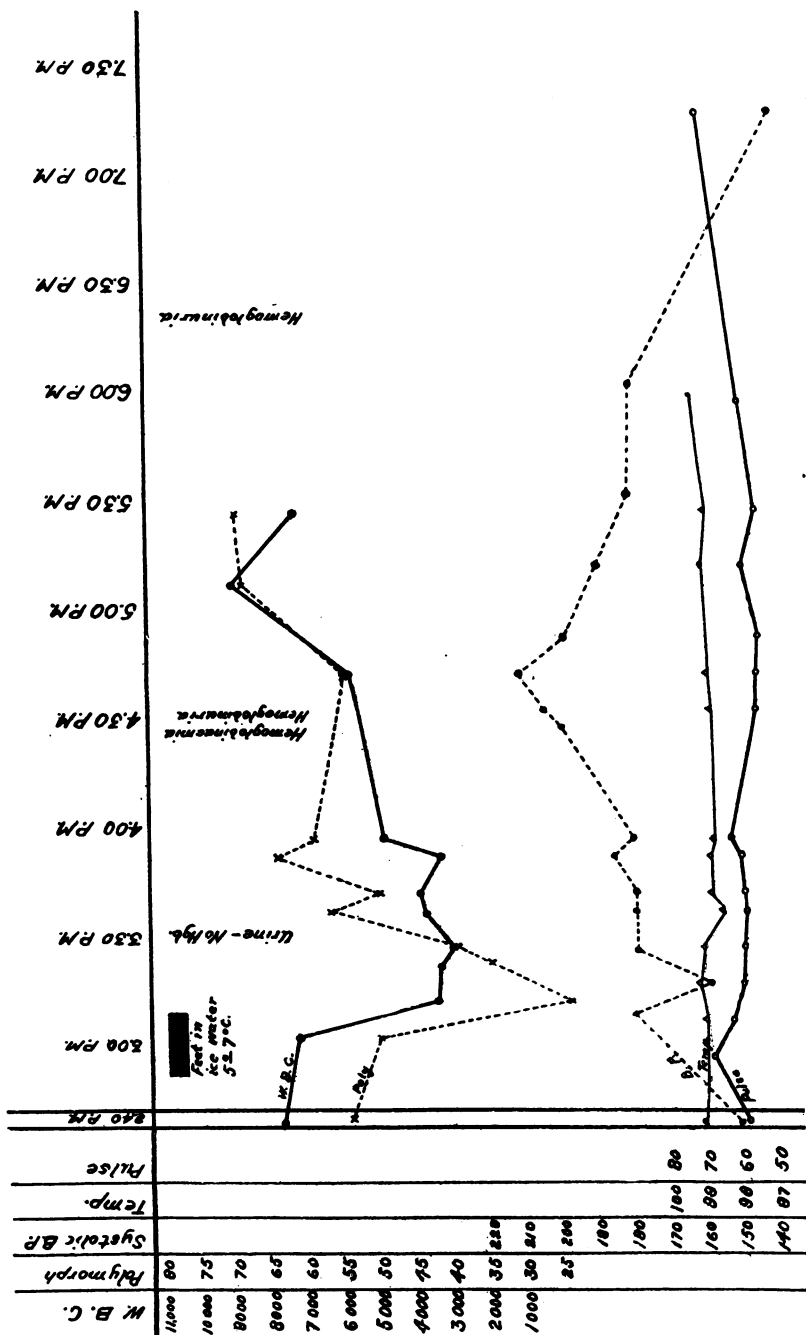


CHART 1. PATIENT J. R. (APRIL 27, 1922.) ARTIFICIALLY INDUCED ATTACK OF HEMOGLOBINURIA

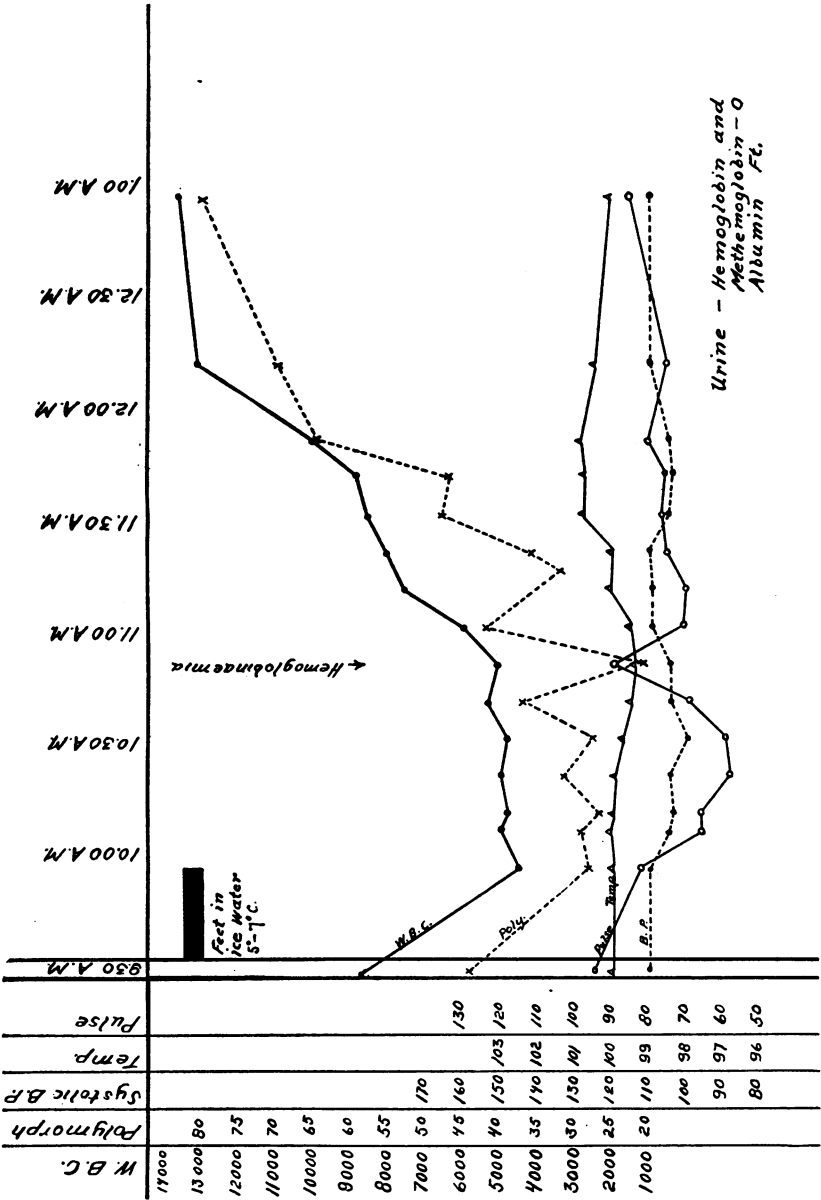


CHART 2. PATIENT J. B. (JULY 11, 1923.) ARTIFICIALLY INDUCED HEMOGLOBINEMIA AND ALBUMINURIA. NO HEMOGLOBINURIA. EXAMPLE OF A LARVAL ATTACK

been done in two ways. The auto-hemolysin was completely absorbed out of hemoglobinuric serum and the Wassermann reaction titrated before and after the absorption of the hemolysin (protocol, 4). Such an experiment shows quite regularly that the auto-hemolysin may be

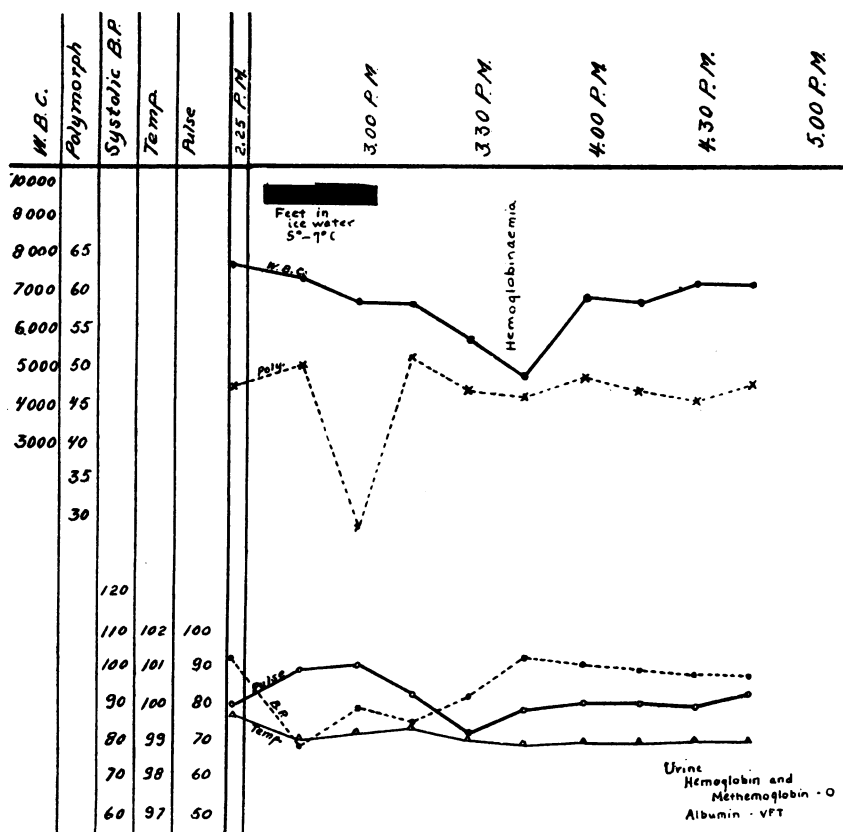


CHART 3. PATIENT G. C. (DECEMBER 20, 1926.) ARTIFICIALLY INDUCED HEMOGLOBINEMIA AND ALBUMINURIA. NO HEMOGLOBINURIA. EXAMPLE OF A LARVAL ATTACK

completely removed without perceptibly weakening the Wassermann reaction. The same result is obtained if hemoglobinuric serum is allowed to stand in the ice-box until the auto-hemolysin has disappeared (protocol 6). It will be found that the Wassermann reacting

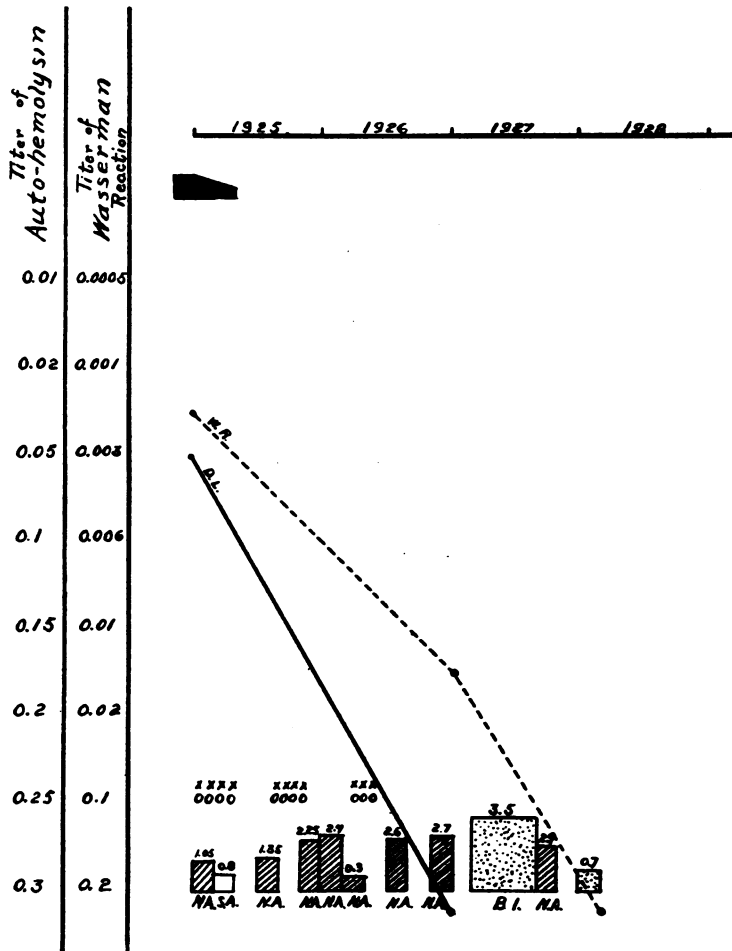


CHART 4. EFFECT OF ANTISYPHILITIC TREATMENT ON AUTO-HEMOLYSIS, W. R. AND SYMPTOMS. PATIENT G. C.

The period during which spontaneous attacks of hemoglobinuria occurred is shown in black at the top of the chart. At the bottom of the chart the kind and amount of treatment is indicated. A = arsphenamine. NA = neoarsphenamine. SA = sulpharsphenamine. BI = intramuscular and intravenous injections of bismuth. The amount of each of these drugs given is shown in grams at the top of the columns representing the different drugs. XXX = inunctions of mercury or intramuscular injections. OOO = potassium iodide. Line WR = Wassermann reaction. Line DL = Donath-Landsteiner reaction.

substance shows no significant change. Furthermore, comparison of the titers of the two reactions in a series of patients shows that the titers do not parallel each other. Such results were perhaps to be

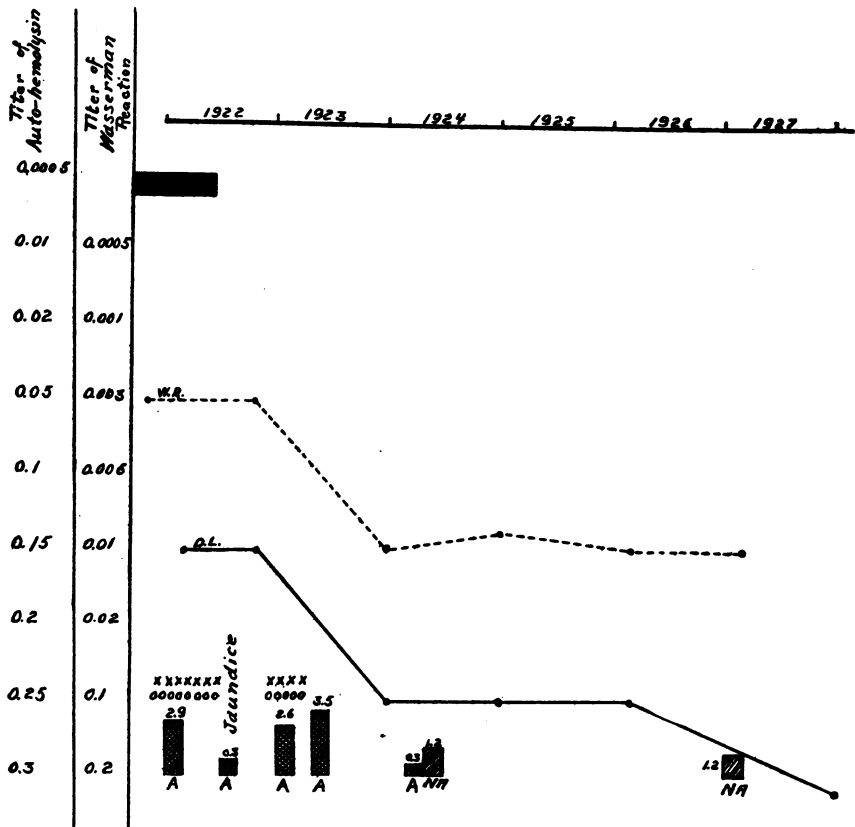


CHART 5. EFFECT OF ANTISYPHILITIC TREATMENT UPON AUTO-HEMOLYSIN, W. R. AND SYMPTOMS. PATIENT L. O.  
Symbols as in Chart 4

expected from the dissimilarity of the effects of temperature upon the two substances.<sup>2</sup>

<sup>2</sup> Burmeister reported experiments in which he found that absorption of paroxysmal hemoglobinuria serum by erythrocytes removed both the auto-hemolysin and the Wassermann reacting substance. In view of the opposite results of Moro and Noda (12), Matsuo (10) and Kaznelson (13) and those reported in this paper, Burmeister's conclusions should not be accepted without confirmation.

Certain very striking vasomotor phenomena are often observed during paroxysms of this disease (14). Two of the five patients had Raynaud's syndrome during attacks; one of these was found to have a systolic blood pressure of 250 mm. Hg. during a spontaneous paroxysm. His usual systolic pressure was 140 to 150 mm. Hg.

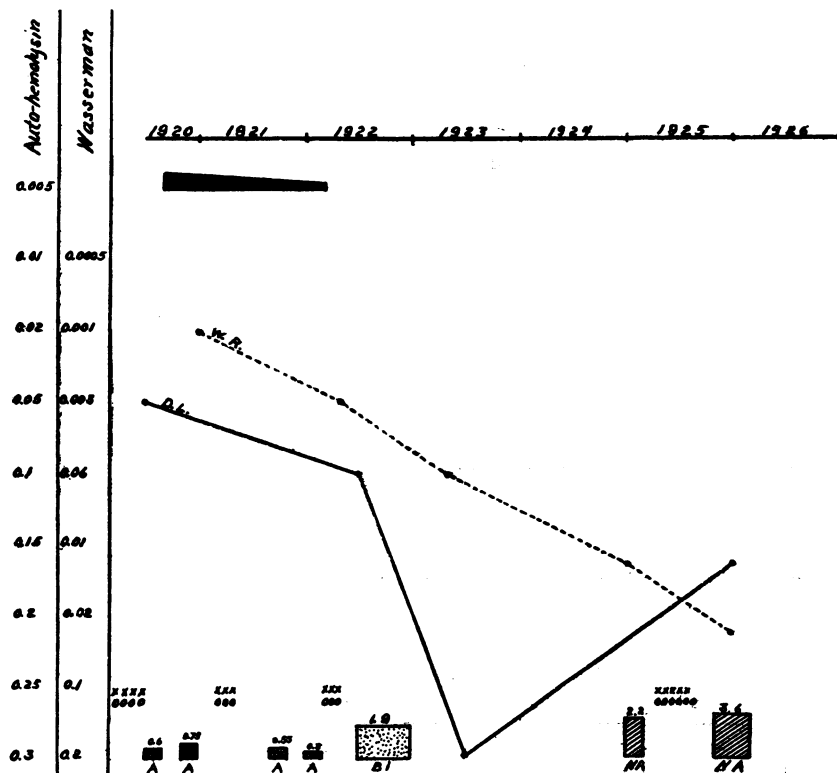


CHART 6. EFFECT OF ANTISYPHILITIC TREATMENT UPON AUTO-HEMOLYSIN, W. R. AND SYMPTOMS. PATIENT J. B.  
Symbols as in Chart 4

When a paroxysm of hemoglobinuria is artificially induced (Rosenbach test) by immersion of the hands or feet in ice water there may be, in addition to hemoglobinemia and hemoglobinuria, changes in blood pressure, temperature and the leucocyte count, the crise hemoclasique of Widal. Chart 1 shows the results of such an experiment. The

TABLE 1  
Summary of clinical, serological and therapeutic observations on 5 patients with paroxysmal hemoglobinuria

Case	Duration	Seasonal occurrence	History relating attacks to chilling	Wassermann reaction	Clinical evidence of syphilis	Vasomotor phenomena	Susceptibility to attacks	Donath and Landsteiner		Rosenbach test	Hemoclastic reaction	Kind of treatment	Result of treatment	Blood group	Remarks
								Result	Titre						
J. B. (F.) Age 9	4½ years	Fall, winter, spring	+	+++++	Hutchinson teeth, enlarged liver	None	++	+	0.05	=	+	Anti-luetic	Cessation of attacks. Donath and Landsteiner and Wassermann reaction still positive	A	
L. O. (M.) Age 58	1 year	Winter	+	+++++	Enlarged tender and irregular liver	None	++	+	0.15	+	=	Irregular anti-luetic	Cessation of attacks. Donath and Landsteiner and Wassermann reaction still positive	A	Donath and Landsteiner later negative. Wassermann reaction still positive
J. R. (M.) Negro Age 40	1½ years	Fall, winter, spring	+	+++++	None	Raynaud's syndrome	+++	+	0.07	+	+	Very irregular anti-luetic	No effect	O	Died lobar pneumonia. Type III. 1926
G. C. (M.) Negro Age 8	6 weeks	Winter	Indefinite	+++++	Hutchinson teeth?	None	+++	+	0.003	=	+	Intensive anti-luetic	Prompt cessation of attacks. Later Donath and Landsteiner and Wassermann reaction negative	AB	
F. V. (M.) Age 7	4 years	All the year, more in winter	+	+++++	Saddle nose Hutchinson teeth, large liver and spleen, anemia	Hives, Raynaud's syndrome	+++++	+	0.2	Not done	Not done	Irregular anti-luetic	General condition improved. Attacks continued. Donath and Landsteiner and Wassermann reaction positive	O	

total white count and the percentage of polymorphonuclears show an abrupt drop after the feet have been in ice water for a few minutes. Synchronous with the decrease in polymorphonuclear cells there is an increase in the percentage of lymphocytes. After one to two hours the leucocytes return to normal, and this may be followed by a leucocytosis due to an increase above normal of the polymorphonuclear cells (chart 2). The blood pressure may be unaffected or may show a gradual increase over a period of about two hours following the chilling (chart 1). Charts 2 and 3 are examples of larval attacks artificially induced. In these experiments hemoglobinemia occurred but there was no hemoglobinuria. Nevertheless, the characteristic changes in the blood picture were observed.

The five patients on whom observations have been made were all given anti-syphilitic treatment (15). Two of these patients did not return with sufficient regularity to receive adequate treatment. The results of treatment on the other three patients are shown in charts 4, 5, and 6. It is perhaps noteworthy that the patient (G. C.) receiving the most intensive treatment showed the most prompt cessation of symptoms and the earliest disappearance of the Donath and Landsteiner and the Wassermann reactions, even though the Donath and Landsteiner had a higher titer in this patient than in any other patient of the series.

Table 1 summarizes some of the clinical, serological and therapeutic observations on the group of patients studied.

#### SUMMARY

Paroxysmal hemoglobinuria is a manifestation of late syphilis; it is characterized by the presence of an extremely labile serum auto-hemolysin which is distinct from the Wassermann reacting substance. Union of the hemolysin with the red blood cells occurs only at low temperatures; short chilling is more effective than long periods at low temperatures; the auto-hemolysin and the iso-hemolysin are probably identical.  $\text{CO}_2$  has been reported to act as a substitute for chilling but such an effect was not demonstrable in the patients of this series. Artificial production of a paroxysm causes hemoglobinemia with or without hemoglobinuria, frequently a sudden drop in the total white count and the percentage of polymorphonuclear cells with a subsequent



leucocytosis and increase of the percentage of polymorphonuclears, an elevation of blood pressure and a rise in the temperature. Intensive anti-luetic treatment may be expected to cause cessation of symptoms, disappearance of the auto-hemolysin and conversion of a positive to a negative Wassermann reaction in the order named.

#### TECHNIQUE

Our routine method of doing the Donath-Landsteiner reaction has been to draw blood from an arm vein, allow it to clot at room temperature, to separate the serum by centrifugalization as soon as coagulation was complete and to set up the test immediately. In a few instances the serum was kept in the ice box over night and the test set up 18 to 24 hours after drawing the blood. The rapid deterioration of the lysin of some of these patients even at ice box temperature, however, makes it advisable to set up the test as soon as the serum has been separated. A 5 per cent suspension of red blood cells was used unless there was a special reason for a heavier or lighter suspension. The suspension was prepared by washing citrated blood three times with a large volume of 0.85 per cent NaCl and suspending the packed cells in 0.85 per cent NaCl. Owing to the thermolability of some of these lysins the serum was used without heating, but to insure the presence of adequate complement fresh pooled guinea pig serum diluted 1:10 was added. The volume was made up to 0.5 cc. with 0.85 per cent NaCl. The chilling was accomplished by placing the tubes in finely crushed ice. The usual time for chilling has been 10 minutes. The warming of the tubes was done by placing them in a water bath at 37°C.

Six protocols illustrating the technical procedures followed in studying some of the questions referred to in the text are included. These are representative of a large number of similar experiments performed during the period of nine years that the patients of this series have been studied.

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