

## PAROXYSMAL HEMOGLOBINURIA

AN EXPERIMENTAL STUDY OF A CASE\*

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In the case presented in this article, on which our work was performed, any severe chilling of the patient's skin caused a temporary hemoglobinuria, drowsiness, regurgitation of food and other minor symptoms to be mentioned later. The phenomena were produced either by a chilling of the general body surface or, what was more common, by the mere exposure of the hands and face to cold, damp winds, or by the wetting of the feet in cold weather. The phenomena were produced experimentally in the laboratory by dipping the patient's feet in water reduced to a temperature of 9 to 12 C., even when the general room temperature was 27 C.

Donat and Landsteiner<sup>1</sup> in 1904 based their explanation of paroxysmal hemoglobinuria on Ehrlich's side-chain theory. Eason,<sup>2</sup> in his admirable paper on this disease in 1906, explained the phenomena in the same way. Hoover and Stone<sup>3</sup> also have shown that the hemolysis preceding the hemoglobinuria may be explained by the side-chain theory. The amboceptor becomes attached to the red blood cells on exposure of the blood to a temperature of 5 to 15 C. for fifteen minutes to one hour. The complement then causes hemolysis when the temperature of the blood is subsequently raised to body temperature for a few minutes. This experiment may be performed in a test-tube by using defibrinated blood.

Hoover and Stone have shown that, in the above experiment, the hemolysis may be prevented by the addition of cholesterin to the defibrinated blood. They have explained this by supposing that the amboceptors attach themselves to the free cholesterin instead of to the red cells. They have supposed that it is the cholesterin of the red cells to which the

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1. Donat and Landsteiner: *München. med. Wehnschr.*, 1904, li, 1590.

2. Eason: *Jour. Path. and Bacteriol.*, 1906, ii, 167.

3. Hoover, C. F., and Stone, C. W.: *Paroxysmal Hemoglobinuria; Account of Two Cases*, *THE ARCHIVES INT. MED.*, 1908, ii, 392.

amboceptors attach in causing hemolysis. Lecithin had no inhibitory effect when used in place of the cholesterin.

Hoover and Stone administered cholesterin to their patients by mouth but were unable to state definitely that any benefit was produced, because their patients would not submit to further experimentation. Their patients had no subsequent attacks of hemoglobinuria but as the weather was moderate during this time no hemoglobinuria would be expected and the patients would not allow its production by means of an iced foot-bath.

Hoover and Stone were able to show, however, that calcium lactate would not inhibit the hemolysis produced by exposure to cold. This result was obtained whether the salt was given by the mouth, in doses of 60 grains daily, or was added to the blood in a test-tube.

Manwaring<sup>4</sup> has shown that calcium chlorid, as well as other salts, inhibits the hemolysis of dog's corpuscles by goat's serum after the goat has been immunized against the dog's corpuscles.

As will be shown later, we have found in our patient that calcium chlorid behaves somewhat like cholesterin in that it inhibits this hemolysis in the test-tube. Like Hoover and Stone we are unable to give definite conclusions after administration of the salt by the mouth because of the refusal of the patient to be experimented on further.

#### HISTORY OF CASE

*Patient.*—F. N. S., aged 28, born in Rochester, N. Y., of German parentage, lived there twenty years, two years in Ohio, three years in Texas, and three years in St. Louis. He has always been in railroad work; is a switchman now. He has been married two years; has no children. His father died at 55 of dropsy of the heart; his mother is alive at 68 in good health. He has five brothers all in good health. One sister had "red urine" fifteen years ago; she had an operation (supposedly for the "red urine") and now enjoys good health. Two other sisters have always enjoyed good health. Our patient had "typhoid-pneumonia" in Rochester; no sickness in Ohio. He had typhoid in Texas in 1904. He lived in St. Louis a year before he had an attack of hemoglobinuria. He has had four attacks of gonorrhea, the last one in 1905. He had warts on the penis in 1904—probably chancroids. The patient eats regularly; his appetite is good. He sleeps well; smokes much; does not drink alcoholic beverages. He has never had an attack of hemoglobinuria in the summer.

*Present Illness.*—The first attack occurred in the fall of 1907 and consisted of one red urination after a chilling of the skin. Recurrences then followed every two or three weeks during the winter of 1907-08 or oftener, depending on the weather. An attack usually began with a chilly sensation. (Hoover and Stone observed a subnormal temperature at the beginning of an attack, and we obtained it once experimentally). This chilling was followed by more or less vomiting, probably a simple regurgitation of food such as we noticed in our experiments. There was no accompanying nausea or pain. Dark, red urine was passed for five to twelve hours afterward. There was much drowsiness, slight pain in the joints

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4. Manwaring: Jour. Infect. Dis., 1904, i, 122.

on movement, particularly of the ankles. The skin of the face, hands or feet (that part exposed to the most cold) first showed a blanching, followed by edema and finally by hyperemia, with a return to normal in ten to twelve hours. A good night's rest in bed was sufficient to cause the patient to feel perfectly well except, possibly, for a slight drowsiness the following day.

*Examination.*—In November, 1908, the patient came under the care of one of us (Neilson) in St. Mary's Hospital, in St. Louis. A thorough physical examination at this time proved practically negative. The blood was examined as to the number of red and white cells, which were normal. No parasites were found. Examinations of the stomach and feces proved negative. Repeated examinations of the urine showed an intermittent albuminuria. The time of the occurrence of albumin was independent of the presence of hemoglobin. Sometimes the two substances occurred together. The experimental application of three small pieces of ice at points about one and one-half inches apart on the back of the hand caused three local areas of edema, which became confluent, then hyperemic, and finally disappeared in six to eight hours. This slight chilling did not cause hemoglobinuria (at least not to a degree appreciable to the eye).

At this time heavy woolen underclothing, felt boots and general instructions to keep warm were given the patient. Ten grains of calcium chlorid three times daily were prescribed. The patient worked all of the winter of 1908-09 with only two very mild attacks of hemoglobinuria. One of these occurred after the patient had temporarily stopped taking the calcium chlorid for a week. The calcium chlorid caused no gastric disturbances.

#### EXPERIMENTS

In July, 1909, the following experiments were performed, the patient continuing to work as switchman for ten to fourteen hours daily. Because of this, and his reluctance to be experimented on, our results are not as complete as we could desire.

*General Methods.*—In determining the hemolytic power of the patient's serum, 10 c.c. of blood were drawn from the median basilic vein and were allowed to clot in a sterile centrifuge tube. The blood was later centrifuged and the serum constitutes the "h. s." (hemolytic serum) of the experiments. A 20 per cent. suspension of washed blood corpuscles was obtained by allowing 1 c.c. of blood from the finger of the patient to run into a few drops of sterile 1 per cent. potassium oxalate solution. The corpuscles were then washed eight times with sterile eighth-molecular sodium chlorid solution by the aid of the centrifuge. The suspension was then diluted with sterile eighth-molecular sodium chlorid to make a 20 per cent. suspension of corpuscles. The calcium chlorid and all glassware used in these experiments were carefully sterilized. In the experiments of July 28, it will be noticed that washed corpuscles were obtained from two sources; one was the patient himself, the other was another man. In the experiments of July 16, only the latter were used. It will be noticed that in the experiments of July 28 slightly different results were obtained with the two different suspensions of corpuscles.

In the experiments of July 19 and 28, the hemoglobinuria was produced experimentally by immersing the patient's feet in water kept at 9 to 12 C. by means of ice. The ice-water was constantly thrown up over the patient's ankles. On July 19, this iced foot-bath was continued for twenty minutes, on the 28th for thirty minutes. On both days the temperature of the room was about 27 C.

*July 16, 1909.*—I. *Experiments Determining the Inhibitory Effect of Calcium Chlorid on the Hemolysis of Red Blood Corpuscles by the Patient's Serum.*—No

calcium chlorid had been given internally to the patient for six months. Ten cubic centimeters of blood were drawn from the patient at 8 a. m. This was allowed to clot for two hours, was then centrifuged. The serum obtained constitutes the "h. s." of the experiments. One cubic centimeter of blood was drawn from another person at 8:30 a. m. The corpuscles were washed eight times with sterile salt solution and were then diluted to 20 per cent. of their original volume. These constitute the "b. c." of the experiments.

The mixtures shown in Table I were made in small test-tubes (3 c.c.) at 11 a. m.

TABLE 1.—HEMOLYTIC POWER OF PATIENT'S SERUM BEFORE TAKING CALCIUM CHLORID

1	1 c.c. m/8 NaCl + 0.2 c.c. h.s. + 0.5 c.c. 20 per cent. b.c.	Placed immediately at 37 C. There was no laking until 4:30 p. m. and then it was slight.
2	1 c.c. m/8 NaCl + 0.2 c.c. h.s. + 0.5 c.c. 20 per cent. b.c.	Placed immediately at 3 C. for 30 min., then at 37 C. After 20 min., at 37 C. there was slight hemolysis. At 1 p. m. there was complete hemolysis.
3	0.3 c.c. m/8 NaCl + 0.2 c.c. h.s. + 0.5 c.c. 20 per cent. b.c. + 0.7 c.c. CaCl <sub>2</sub>	Placed at 37 C. (as No. 1). There was slight hemolysis at 3 p. m. (possibly of bacterial origin).
4	0.3 c.c. m/8 NaCl + 0.2 c.c. h.s. + 0.5 c.c. 20 per cent. b.c. + 0.7 c.c. CaCl <sub>2</sub>	Placed at 3 C. for 30 min., then at 37 C. (as No. 2). There was slight hemolysis at 3:30 p. m.

At 4:30 p. m. tubes 1, 3 and 4 looked alike; there was slight hemolysis in all. In tube 2 there was complete hemolysis.

This experiment was performed a second time. At both trials varying amounts of hemolytic serum and of calcium chlorid were used, but without changing the results.

The first point to be noted in this experiment is that, in Mixtures 1 and 3, which were not subjected to the low temperature of 3 C., but which were placed immediately at 37 C., there was only slight hemolysis, and this occurred only after four hours. This may have been due in part to bacterial action; what is more probable, however, is that there was sufficient cooling of the blood from 8 a. m. to 11 a. m. to cause some hemolysis to occur after the blood was placed at 37 C. From the time the blood was drawn (8 a. m.) to the time the mixtures were made (11 a. m.) the patient's serum was at room temperature, which was 27 C. to 28 C. It was impossible to keep the blood at body temperature all of this time.

The second point to note is that hemolysis did occur readily in Tube 2. This tube was subjected to a temperature of 3 C. for thirty minutes before it was placed at 37 C. Theoretically it was during the exposure to 3 C. that the amboceptors became united to the red cells, the complements causing the laking when the blood was raised to 37 C. for a few minutes.

The third point to note is that in Tube 4, the hemolysis was inhibited by the presence of 0.7 c.c. of m/8 CaCl<sub>2</sub> (in place of so much NaCl), although this tube was subjected to the same cooling and subsequent warming as was Tube 2 (in which there was complete laking).

*July 19, 1909.—II. Experiments Determining the Effect of the Iced Foot-bath (Applied for Twenty Minutes) on the Patient.* No calcium chlorid had been given internally to the patient for six months

TABLE 2.—BLOOD-COUNT AFTER ICED FOOT-BATH—NO CALCIUM CHLORID

Time.	Body temp.	Urine	Blood Corpuscles						
			Red		White				
			Absolute.	Absolute.	Relative (%)				
					Poly.	L.L.	S.L.	M.	Eos. Mono.
8:30 a. m.	98.4	Normal	5,200,000	11,520	62	23	12	2	3 0
8:40 to 9 a. m.		Iced foot-bath.	Regurgitation of food at the end of time.						
9:00 a. m.	97.8	Dark	5,680,000	10,100	62	23	8	1	3 3
11:10 a. m.	98.7	Black	5,760,000	10,600	90	6	4	0	0 0
2:30 p. m.	98.0	Brown	5,120,000	8,600	80	19	1	0	1 0

The first point to which we wish to call attention is the apparent health of patient at 8:30 a. m.

The second point is the regurgitation of food at the end of the iced foot-bath, the subnormal temperature, the hemoglobinuria and the very slight change in the absolute number of red and white blood corpuscles. We have no explanation to offer for the changes in percentage of the white corpuscles. Careful differential counts were made by two different people—their results tallying—on different smears.

The third point is the other symptoms; the ankles first became edematous then hyperemic, returning to normal in about three hours. The patient became drowsy. The hemoglobinuria lasted for six hours. Patient felt as well as ever the following day.

July 28, 1909.—III. *Experiments Determining the Effect of the Internal Administration of Calcium Chlorid for Eight Days.*—Immediately after the experiments of July 19, the patient was given ten-grain doses of calcium chlorid three times a day for eight days. These experiments are divided into two parts:

1. At 8 a. m. 10 c.c. of blood were drawn from the median basilic vein. This blood was allowed to clot, the serum being used to determine the effect of the calcium chlorid on the hemolytic power in the test-tube. These experiments were similar to those performed on July 16 (before any calcium chlorid had been given to the patient). In these experiments we attempted to find whether there had been sufficient absorption of the salt into the blood to cause inhibition of the hemolysis.

2. At 8:37 a. m. the patient was given an iced foot-bath similar to the one of July 19, except that in this case the bath was continued for thirty minutes instead of twenty minutes, because at the end of twenty minutes the patient showed no signs of an attack of hemoglobinuria and said he was not going to have an attack. As is shown below, we did cause a mild attack by prolonging the bath.

1. Results of the experiments determining the hemolytic power of the patient's serum in the test-tube. These experiments were performed exactly as were those on July 16. In these, however, two different samples of corpuscles were used. Mixtures 1, 2 and 3 were made using the patient's own red blood cells, mixtures 4, 5 and 6 were made with corpuscles from the subject used for this purpose on the 16th. The blood was drawn at 8 a. m. The mixtures were made at 11:30 a. m.

TABLE 3.—HEMOLYTIC POWER OF PATIENT'S SERUM AFTER TAKING CALCIUM CHLORID.

1	0.5 c.c. m/8 NaCl + 0.4 c.c. h.s. + 0.25 c.c. b.c.	Placed immediately at 37 C. No hemolysis at 5 p. m.
2	0.5 c.c. m/8 NaCl + 0.4 c.c. h.s. + 0.25 c.c. b.c.	Placed at 3 C. for one hour, then at 37 C. Slightly laked at 1:30 p. m. No more at 5 p. m.
3	0.5 c.c. m/8 CaCl <sub>2</sub> + 0.4 c.c. h.s. + 0.25 c.c. b.c.	Placed at 3 C. for one hour, then at 37 C. No hemolysis at 5 p. m.
4	0.5 c.c. m/8 NaCl + 0.4 c.c. h.s. + 0.25 c.c. b.c.	Placed immediately at 37 C. No hemolysis at 5 p. m.
5	0.5 c.c. m/8 NaCl + 0.4 c.c. h.s. + 0.25 c.c. b.c.	Placed at 3 C. for one hour, then at 37 C. Complete hemolysis at 1:30 p. m.
6	0.5 c.c. m/8 CaCl <sub>2</sub> + 0.4 c.c. h.s. + 0.25 c.c. b.c.	Placed at 3 C. for one hour, then at 37 C. No hemolysis at 5 p. m.

The first point to be noted in this experiment is that not a sufficient amount of the calcium chlorid had been absorbed into the blood to inhibit completely the hemolysis in either tube 2 or 5.

The second is that there was more hemolysis with the foreign corpuscles (5) than when the patient's own corpuscles were used (2). These results were obtained when the blood serum was cooled to 3 C. for one hour.

The third point is that the hemolysis produced by the exposure to the temperature of the 3 C. was completely inhibited in Tubes 3 and 6 to which calcium chlorid was added in place of the sodium chlorid.

Fourth, another obvious point is that the hemolysis did not occur in those tubes (1 and 4) which were not subjected to the cooling (3 C.), but which were placed immediately at 37 C.

2. Results of giving the patient an iced foot-bath; the principal symptoms are briefly summarized in Table 4.

TABLE 4.—BLOOD-COUNT AFTER ICED FOOT-BATH, FOLLOWING CALCIUM CHLORID

Time.	Body temp.	Urine.	White Blood Cells, Relative (%)					
			Poly.	L.L.	S.L.	Mast.	Eos.	L. Mono.
8:30 a. m.	98.8	Normal	(Lost)					
8:37 to 9:07.	Iced foot-bath.	No regurgitation of food.						
9:10 a. m.	98.6	Brownish	68	26	4	0.5	0.5	1
10:10 a. m.	98.6	Normal	25	65	8	1	1	0
3:30 p. m.	98.6	Normal	77	17	0	2	2	2

The points to which we wish to call attention are, first, the apparent health of the patient at 8:30 a. m.; second, the longer time during which the foot-bath was given over that of July 19; and, third, the fact that the ankles did not show the edema that they did on July 19. There was no regurgitation of food, no sub-normal temperature, and only slight hemoglobinuria for one urination. We have no explanation for the variations in the percentage of the white blood cells. The patient did not become drowsy. He said, after the bath that he felt as well as he ever did.

A long course of the internal administration of calcium chlorid was then attempted, but furunculosis occurred and the salt was discontinued after two months. No hemoglobinuria occurred during this time; the weather was not severe, however, and the patient refused to be further experimented on.

We cannot state definitely that calcium chlorid when administered by the mouth does prevent this hemoglobinuria, but in view of the inhibitory effect on hemolysis in the test-tube, and the apparent greater difficulty of producing hemoglobinuria by foot-baths after the administration of calcium chlorid, we feel justified in concluding that if sufficient calcium chlorid could be introduced into the blood, and retained, no hemoglobinuria would occur.

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