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Cold Agglutinated Erythrocytes: Hemolytic Effect of Shaking.

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The influence of mechanical injury upon the fragility of erythrocytes has not received much attention. The observations reported in this communication indicate an unusual effect of shaking upon cold agglutinated erythrocytes.

In a detailed study Salén[1] has clearly shown that hemolysis of cold agglutinated erythrocytes may occur under certain conditions in the absence of complement. He stressed the fact that the hemolysis differed from the cold-warm hemolysis of Donath and Landsteiner[2] described in syphilitics and in patients with syphilitic cold paroxysmal hemoglobinuria. Other writers (cited in [3]) have commented on the occurrence of hemoglobinemia and hemoglobinuria in cases of cold hemagglutination.

Materials and Methods. Recently observed cases (cited in [3] and [4]) of extremely high titers of cold hemagglutinins have been studied for hemolysis by means of the tests about to be described. In each case hemoglobinemia was readily demonstrated by immersion of an extremity in cold water.

The first was a patient with acute hemolytic anemia without hemoglobinuria during the course of an atypical bronchopneumonia. Six grams of sulfadiazine had been administered 4 days before the apparent onset of the anemia. At the height of the disease the cold hemagglutinin titer was 1/20,000 at 4° C. At this time the *in vitro* hemolytic phenomena, to be described below, were positive. Thirteen days later, when the titer had fallen to 1/800 at 4° C, hemolysis could not be demonstrated.

The second case[4] was a patient with symmetrical gangrene of the tips of the fingers and toes and hemoglobinuria after exposure to moderate environmental cold. The cold hemagglutinin titer of 1/30,000 at 4° C in this instance was constant over many months; the *in vitro* hemolytic tests were always positive.

The experiments were performed within 3 hours of the collection of the blood, except the old and old lyophylized serums mentioned later. Blood or serum was stored at 37° C prior to use. Inactivated serum was heated at 56° C for 1½ hours before use. The old serum* had been collected aseptically 15 months before its use and stored at 6° C. The old lyophylized serum* was also collected 15 months and lyophylized 14 months prior to the experiments. It was made up to its original volume with distilled water before use.

"Blood of the patient" in the experiments refers to the previously mentioned cases with cold hemagglutinins. In all tests homologous or heterologous Group O erythrocytes previously washed with 0.85% sodium chloride solution were employed. The dilutions of the patients' serums were made with 0.85% sodium chloride solution or with normal serum of the same blood group.

All experiments were performed in 10x75 mm serological tubes. The tests were set up and the reactants mixed gently at 37° C. Shaking of the tubes was carried out in a standard manner at room temperature; the tube was held upright at the top between the thumb and index finger of one hand and was tapped at the bottom lightly in rapid succession with the index finger of the other hand. This manipulation was completed in 10 seconds and the tube was replaced in the rack at the temperature of that particular experiment.

[1] Salén, E. B. *Acta med. Scandinav.*, 1935, **86**, 570.

[2] Donath, J., and Landsteiner, K., *Ergeb. d. Hyg., Bakteriol. und exp. Therap.*, 1925, **7**, 184.

[3] Stats, D., and Wasserman, L. R., *Medicine*, in press.

[4] Stats, D., and Bullowa, J. G. M., *Arch. Int. Med.*, 1943, **72**, 506.

* These serums were collected and prepared in the Littauer Pneumonia Laboratory, Harlem Hospital, New York City, under the direction of Dr. Jesse G. M. Bullowa.

In some of the tests the final readings of hemolysis were made after sedimentation or centrifugation at the temperature of the experiment; in others the results were read after centrifugation at 700 r.p.m. for 2 minutes at room (22°C) temperature.

The term "marked hemolysis" refers to a coloration equivalent to that produced by the complete hemolysis of a 4 to 6% suspension of red blood cells. Since the final erythrocyte concentration was as high as 40% in some experiments (whole blood), hemolysis was never complete.

Results. Summaries of typical experiments which elucidate the mechanism of hemolysis with cold hemagglutination follow:

1. .3 cc of fresh, heat inactivated, old or old lyophylized serum of the patient was mixed with .2 cc 80% suspension of R.B.C. (This was actually a 32% erythrocyte suspension in the final mixture). After incubation at 4°C for 30 min., during which time the tubes were tapped 20 times every 5 min., the tubes were centrifuged. Marked hemolysis was observed.

2. The same experiment as above performed at 4°C without tapping or at 37°C with tapping failed to result in hemolysis. There was marked hemagglutination at 4°C.

3. The same experiment as in (1) carried out with blood serum from normal individuals or from individuals with cold hemagglutinins of titer lower than 1/2500 did not result in hemolysis.

4. The hemolysis of experiment (1) persisted, but in diminishing degree, through a serum dilution of 1/8 and a dilution of the red blood cell suspension of 10% (4% R.B.C. in the final mixture). More dilute serum or red blood cell suspensions did not reveal hemolysis though cold hemagglutination was still marked.

5. .5 cc oxalated whole blood of the patient was kept at 4°C for 30 seconds. At the end of this time the tube was tapped 20 times. No hemolysis was observed after centrifugation.

6. .5 cc oxalated whole blood of the patient was kept at 4°C for 90 seconds. At the end of this time the tube was tapped 20 times. Marked hemolysis was observed.

7. .5 cc oxalated whole blood of the patient was kept at 4°C for 5 minutes. At the end of this time the tube was tapped 2 times. Moderate hemolysis was observed.

8. .5 cc oxalated whole blood of the patient was kept at 4°C for 30 minutes without tapping. The tube was then transferred to the water bath at 37°C. After one minute at this temperature, the tube was tapped 20 times. The tapping was repeated at 5-minute intervals for 30 minutes. After another 30 minutes had elapsed, the tube remaining at 37°C, the tube was centrifuged. No hemolysis was present.

These data demonstrate the existence of a unique type of hemolysis. Concentrated erythrocyte suspensions subjected to marked cold agglutination are readily hemolysed by shaking or tapping. The amount of shaking is not sufficient to hemolyse relatively weakly agglutinated or non-agglutinated erythrocytes. No hemolysis was observed in cases in which the cold hemagglutinin titers were lower than 1/2500 at 4°C. Similarly, light erythrocyte suspensions, even though strongly agglutinated in the cold, were not hemolyzed. Of special interest is the occurrence of hemolysis in the absence of complement. This phenomenon, therefore, is not entirely immunological for lysis requires shaking of the cells but not complement. Hemolysis of cold agglutinated erythrocytes is different from the hemolysis of the Donath-Landsteiner test. In the latter, hemagglutination is slight; erythrocytes sensitized in the cold by amboceptor are hemolyzed at 37°C by complement, lysis failing to occur in the cold.

Summary. These experiments demonstrate an unusual type of hemolysis dependent jointly upon a high concentration of erythrocytes, extreme potency of cold hemagglutination and, presumably, damage as the result of shaking.