Production of Positive Antiglobulin Serum Test in Rabbits by Intraperitoneal Injection of Homologous Blood.* (19319)

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Studies of acquired hemolytic anemia indicate that the red cell sensitizing agent is a fraction of plasma protein similar in many ways to isoantibodies, but it is not known what conditions are necessary for its production. Since it appears to be an antibody specific for human red cells, attempts to produce the disease experimentally should include attempts to sensitize the animal to an antigen in autologous red cells. Attempts to produce autoantibodies for red cells are not new. Troisier(1) reported that in hemothorax and hemoperitoneum autohemolysins appeared in the blood and gradually increase in titer. Ludke(2) produced iso- and auto-lysins in 9 of 11 dogs by reinjecting lysed red blood cells which were obtained from the same dog. Recently Wagley and Castle(3) showed agglutination in anti-dog rabbit serum of the red cells of one of 4 dogs which had been injected with a modified Freund antigen red cell mixture. The application of the antiglobulin serum technic(4) in the experimental approach to acquired hemolytic anemia is an advance over former methods since it is the most reliable technic yet devised for detecting sensitization of red cells. Most antibodies found in acquired hemolytic anemia do not produce hemolysis with complement fixation *in vitro* and are usually weak agglutinins even in colloidal media and can be demonstrated only with antiglobulin serum. It may be that antibodies produced in animals by experimental methods will exhibit the same properties.

During a study of a patient with anemia secondary to intraperitoneal bleeding from a ruptured ectopic pregnancy, a transient positive antiglobulin test was observed. In an effort to simulate this occurrence in animal experiments, rabbits were given intraperitoneal injection of their own blood after removal by cardiac puncture. The red cells of the rabbits so injected were tested for sensitization in anti-rabbit guinea pig serum. Other rabbits received injections of rabbit red cell stroma that had been incubated with filtrate from hemolytic streptococcus cultures or with emulsions of rabbit liver or kidney.

Preparation of anti-rabbit globulin guinea pig serum (ARGGS). Five-tenths cc of pooled rabbit serum was injected subcutaneously once a week for 4 succeeding weeks into 4 guinea pigs. A one-week resting period was followed by 4 succeeding weekly injections. Blood was obtained by cardiac puncture or the animal was sacrificed and bled 10 days after the final injection. The serum was separated from the clot and heated to 56°C for 30 minutes and absorption of red cell antibodies was carried out by successive incubation at room temperature with half the volume of pooled red cells from 8 normal rabbits. After two absorptions the serum no longer agglutinated red cells from normal rabbits. The activity of the absorbed anti-rabbit serum was demonstrated by preciptin tests with normal rabbit serum. A positive precipitation reaction was obtained up to the 1-100 dilution of the rabbit serum with a 1-5 dilution of guinea pig serum. Control guinea pig sera gave negative results. Preliminary to injection of the test rabbits their cells were tested and found to be negative to ARGGS.

Intraperitoneal injection of homologous blood in 12 rabbits. A total of 12 rabbits were used. The usual aseptic technics were employed to prevent contamination, and none of the rabbits showed signs of infection. The first group consisted of 4 rabbits, each of which received intraperitoneally 20 cc of whole blood, which was obtained by cardiac puncture once a week for 4 weeks. The blood was injected immediately after withdrawal from the heart without anticoagulant. Once or twice a week during the period of injection

* This work was supported by a grant from the U. S. Public Health Service.

the animal's RBC were tested with ARGGS, and the 28th day after the first injection, the antiglobulin test was positive to a 1/20 dilution in 2 of the rabbits. Injections of red cells were stopped and the positive reaction persisted 14 days. The *second group* consisted of 8 rabbits which received weekly injections for 3 weeks, then biweekly injections for 3 weeks. At the end of the 3rd and 4th week the washed RBC of one rabbit were agglutinated in a 1/5 and 1/10 dilution of antiglobulin serum. No other evidence of immunological abnormality such as the development of auto- or isohemolysins, or agglutinins in normal or acidified serum was demonstrated in the sera of rabbits whose red cells showed a positive antiglobulin serum test. There was no evidence of anemia, reticulocytosis or of increase in osmotic or mechanical fragility.

Injection of red cell stroma plus bacterial filtrate, kidney emulsion or liver emulsion. Red cell protein was collected as stroma in the Sharples centrifuge after hemolysis of washed rabbit red cells in 10 volumes of distilled water. One volume of undiluted B-hemolytic streptococcus filtrate plus 1½ volumes of 5% red cell stroma were incubated at 37°C for 2 hours, then stored at −30°C. Of 14 rabbits 4 received 2.5 cc red cell stroma streptococcus filtrate mixture, 2 received 1.5 cc 5% red cell stroma, and 2 received 1 cc streptococcus filtrate by daily subcutaneous injection for 30 days. Four rabbits received 1.5 cc 5% red cell stroma plus 2 cc rabbit kidney emulsion and 2 rabbits received the same amount of red cell stroma plus 2 cc of rabbit liver emulsion twice a week for 4 weeks by subcutaneous injection. Hemoglobin, reticulocyte count, and antiglobulin tests were done before, during and after the injections for a 3 month period. However, there was no significant change in hemoglobin and reticulocyte count and the red cells continued to show a negative antiglobulin serum test.

Discussion. There is only slender evidence for the postulate that the red cell antibody of acquired hemolytic anemia is a specific response to an antigen in the red cell. Perhaps the best evidence that a foreign substance may form an antigenic complex which results in an antibody capable of destroying the tissue part of the complex are the observations of Ackroyd(5) in sedormid thrombocytopenic purpura. However, the antibody in this case is not active for the thrombocyte alone. It may be that a foreign substance also plays a role in the initiation of acquired hemolytic anemia. It is a clinical impression that there is a high incidence of infection, trauma and medication in the background of patients with the disease.

The observation that intraperitoneal injection of red cells may result in a positive antiglobulin serum test suggests that relatively bland treatment of red cells may result in modification of surface structure sufficient to provoke antibody production. The nature of possible changes in red cell structure produced by intraperitoneal injection is not known, but it is conceivable that tissue enzymes or free fatty acids in lymph may produce significant alterations.

Summary. (1) Three of 12 rabbits receiving intraperitoneal injections of blood immediately after removal from the heart developed a transiently positive antiglobulin serum test (Coombs test) with a reagent made by injecting rabbit serum into guinea pigs. None of the 3 developed evidence of a hemolytic anemia. (2) Rabbits receiving injections of rabbit red cell stroma which had been incubated with bacterial filtrate or emulsions of rabbit liver or kidney failed to develop positive agglutination tests with the same reagent.

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Received January 9, 1952. P.S.E.B.M., 1952, v79.