

Antiglobulin Test in Anemia Caused by Acetylphenylhydrazine. (22822)

VINCENT P. PERNA, DWIGHT W. ROBINSON, AND W. B. STEWART

Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

Following intravenous injection of red cells from one individual to another of the same species, certain sensitizing antibodies may develop. They may be detected *in vitro* by the antiglobulin test (Coombs Test). This test depends upon the sensitization of red cells by such antibodies, with subsequent agglutination of these sensitized cells by antiglobulin serum. At times, autosensitization may occur, as in certain types of acquired hemolytic anemia. It has generally been concluded that, for unknown reasons, the affected individual developed antibodies to his own red cells, which were then destroyed, thus accounting for the anemia. On the other hand, it is possible that a positive Coombs test may be caused by injury of some other type, and may not involve a specific immunological reaction. This was suggested by a report of Muirhead, Groves and Bryan(1) who found a positive antiglobulin reaction in dogs during anemia produced by acetylphenylhydrazine. We have repeated the experiments, but failed, however, to confirm them. A preliminary search to account for the difference in results was unsuccessful so the disparity remains unexplained. Since these findings are of considerable theoretical importance, we feel the data should be recorded.

Methods. The technic of Muirhead, Groves and Bryan(1) was used, and, in addition, certain modifications were introduced in attempts to account for differences in results. Two different types of Coombs serum were prepared. The first (Serum A) was made by injecting whole canine serum into rabbits, exactly as directed(1). Since it seemed possible that the positive reactions reported(1) were due to antibodies other than antiglobulins, a second type of Coombs serum (Serum B) was prepared as follows: the globulin fraction was separated from canine plasma by ammonium sulfate precipitation. An alum precipitate was prepared from this(2). Rabbit antiserum was then produced and ab-

sorbed against pooled canine erythrocytes. The titers of the 2 types of Coombs sera were nearly the same, with identical specificities. On each day, both sera were used, and each was controlled by an indirect Coombs test against the test cells sensitized by a canine isoantibody. The dogs were given a single intravenous dose of 40 mg/kg of acetylphenylhydrazine as directed(1). Direct Coombs tests were performed at frequent intervals thereafter. In addition, in each instance, 2-fold serial dilutions of the Coombs sera were made in an attempt to demonstrate the prozone phenomenon reported(1). In doing these titrations some of the tests were set up using 1 ml of a 3% suspension of test erythrocytes rather than the 0.5 ml of a 2% suspension as was previously reported(1). Dogs 1-4 were done with the former, and dogs 5-8 with the latter. On several occasions both methods were used together and no difference was detected. The criteria for a positive reaction were those used by Muirhead, Groves and Bryan(1). Hematocrit determinations were done in Wintrobe tubes using heparinized venous blood.

Results. The results are summarized in the Table. Since all titrations were negative, only the result of the ordinary direct Coombs test are given. With the exception of one weakly positive test and 2 doubtful tests on the 9th day in dogs 1, 2 and 3, all were clearly negative. It may be of significance that these positive tests were all obtained against the Coombs serum produced against the whole canine serum. The tests using the true antiglobulin serum were uniformly negative. Whether the conflicting results of our experiments and those of Muirhead *et al.* were due to difference in the antiglobulin sera used remains undetermined. Further work along these and similar lines seems necessary.

Summary. No evidence of a positive antiglobulin test was found in dogs following injection of acetylphenylhydrazine. This is in

TABLE I. Reactions of Canine Erythrocytes with 2 Types of Coombs Sera on Representative Days following a Single Intravenous Dose of Acetylphenylhydrazine.

Dog #	1			2			3			4		
Day after phenylhydrazine	Het, %	Serum A*	B†	Het, %	Serum A	B	Het, %	Serum A	B	Het, %	Serum A	B
0	49	—	—	45	—	—	56	—	—	51	—	—
1	41	—	—	34	—	—	51	—	—	44	—	—
4	31	—	—	26	—	—	40	—	—	36	—	—
7	27	—	—	28	—	—	27	—	—	28	—	—
9	30	±	—	31	±	—	34	1+	—	30	—	—
11-14	39	—	—	37	—	—	42	—	—	38	—	—
15-18	42	—	—	37	—	—	43	—	—	41	—	—
19-24	49	—	—	47	—	—	51	—	—	46	—	—

Dog #	5			6			7			8		
Day after phenylhydrazine	Het, %	Serum A*	B†	Het, %	Serum A	B	Het, %	Serum A	B	Het, %	Serum A	B
0	46	—	—	41	—	—	53	—	—	44	—	—
1	37	—	—	36	—	—	42	—	—	23	—	—
4	33	—	—	23	—	—	31	—	—	29	—	—
7	31	—	—	30	—	—	27	—	—	26	—	—
9	33	—	—	30	—	—	27	—	—	25	—	—
11-14	36	—	—	33	—	—	35	—	—	34	—	—
15-18	38	—	—	35	—	—	39	—	—	34	—	—
19-24	46	—	—	46	—	—	44	—	—	42	—	—

* Serum A: Antiserum against whole dog serum.

† Serum B: " " dog globulin (see text).

contradiction to the results of others.

1. Muirhead, E. E., Groves, M., and Bryan, S., *J. Clin. Invest.*, 1954, v33, 1700.2. Kabat, E. A., and Mayer, M. M., *Experimental immunochemistry*, 1948, C. C. Thomas, Springfield, Ill., 543.

Received October 4, 1956. P.S.E.B.M., 1956, v93.

Effect of Russell's Viper Venom (Stypven) on Stuart Clotting Defect. (22823)

CECIL HOUGIE (Introduced by Cornelia Hoch-Ligeti)

Department of Clinical Pathology, University of Virginia, Charlottesville.

In a study of patient (R.S.) with congenital hemorrhagic disorder of moderate severity, Lewis, *et al.*(1) showed he had a prolonged one-stage prothrombin time which was corrected by normal plasma or serum but not by barium sulphate-adsorbed plasma or serum. The 2-stage prothrombin was normal and prothrombin consumption abnormal. These authors believed that the factor deficient in this patient was identical to that previously described by Alexander, *et al.*(2) Koller, *et al.*(3) and Owren(4) and now referred to as SPCA, factor VII or procon-

vertin (the 3 terms being used synonymously). It has now been established that factor VII (SPCA or proconvertin) is not essential for generation of blood thromboplastin(5,6). In a re-study of patient (R.S.) it has been found that the patient's serum is deficient in a factor essential for blood thromboplastin formation(7). Thus if various concentrations of normal serum in the patient's serum are prepared and incubated with normal Al(OH)₃-adsorbed plasma and platelets in the thromboplastin generation test, maximum yield of thromboplastin generated