Activity of C'1 in Rabbit Antisheep Hemolysin. (27214)

L. L. DeLooze, Arnold Ransby and Myron A. Leon (Introduced by M. H. Kaplan)
Department of Surgical Research, St. Luke's Hospital, Cleveland, Ohio

Maurer and coworkers have demonstrated the uptake of protein nitrogen by specific precipitates of antigen and antibody from fresh, aged or heated rabbit serum(1,2). This nitrogen uptake has been, at least in part, ascribed to fixation of components of complement(3). It therefore became of vital concern, in our studies of the reactions of human complement with sheep erythrocytes sensitized by rabbit hemolysin, to ensure the absence of components of rabbit complement from the system. The data presented here demonstrate that erythrocytes sensitized by some samples of rabbit antibody show the activity of C'1, and further, that the C'1 is eluted in the presence of sodium ethylenediamine tetraacetate (Na_3HEDTA).

Materials and methods. Sheep erythrocytes (E) were preserved in Alsever solution containing 0.008 *M* Na_3HEDTA. Rabbit antisheep cell hemolysins (A) were obtained from commercial sources and contained 50% glycerol. The reagents R1 and R4 were prepared by standard technics(3). Barbital buffer containing 0.00015 *M* Ca^{++} and 0.0005 *M* Mg^{++}(3) was used as a diluent unless otherwise specified.

Results and discussion. Washed sheep erythrocytes (1×10^9/ml) were sensitized with a 1:800 dilution of hemolysin. A parallel sensitization was performed with both erythrocyte supension and hemolysin dilution containing 8×10^{-3} *M* Na_3HEDTA. The two kinds of sensitized erythrocytes, labeled EA and EA(EDTA) respectively, were incubated at 37°C for 20 minutes, centrifuged, washed twice and restandardized to contain 5×10^8 cells/ml.

Aliquots (2.0 ml) of these supensions were centrifuged and the sedimented cells mixed in the cold with 4.0 ml portions of either human R1 diluted 1:40 or human R4 diluted 1:20. The mixtures were incubated at 37°C for one hour and the extent of hemolysis is recorded in Table I as optical densities at 541 mμ. It is evident that cells sensitized in the absence of EDTA reacted with rabbit C'1. No evidence for the presence of a significant quantity of C'4 was found with either the human R4 recorded in Table I or with a rabbit R4.

TABLE I. Demonstration of Rabbit C'1 in EA.

	R1	R4
	O.D.	
EA	.450	.046
EA(EDTA)	.019	.032

The amount of C'1 fixed from the 10 antisera tested varied widely. Data for 4 representative antisera are shown in Table II. C'1 in these tests was measured with $MgNa_2$-EDTA treated human serum which provides C'2, C'3 and C'4(4). The C'1 activity, contributed by the rabbit antisera, could be eluted from the EA by extraction with Na_3-HEDTA at 37°C(5) as shown in line 3 of Table II. Control EA, extracted with barbital, retained their C'1 activity.

The heat stability of rabbit C'1 in antiserum A was determined by incubating 0.2 ml portions at 56°C for 30, 45 and 60 minutes. The serum was added to preheated test tubes (18 × 125 mm) to minimize the time necessary to reach 56°C. EA and EA(EDTA) prepared from these and from an unheated sample were tested for reactivity with $MgNa_2$EDTA treated human serum. The results (Table III) show that considerable rabbit C'1 remained in the hemolysin after a one-hour incubation at 56°C.

The presence of appreciable amounts of rabbit C'1 on EA would certainly make them unsuitable for studies of the mechanism of action of human or guinea pig complement. Although rabbit antisera could be screened to eliminate those contributing C'1 activity to EA, all rabbit antisera might contribute hemolytically inactive C'1 since the site of attachment to the cell and the site for hemolytic activity of C'1 might well be different. The routine use of EDTA in preparation of EA would obviate such doubts. However, in view of the report(6,7) that a new com-

TABLE II. Fixation of C'1 from Various Hemolysins.

	Hemolysin A	Hemolysin B	Hemolysin C	Hemolysin D
	O.D.			
EA	1.315	.260	.063	.015
EA (EDTA)	.007	.017	.009	.007
EA (extracted)*	.010	.008	.000	—

* EA incubated at 37°C for 15 min. in presence of .008 M Na_3HEDTA, centrifuged, washed twice and restandardized.

TABLE III. Stability of Rabbit C'1 at 56°C.

	Incubation time			
	0 min.	30 min.	45 min.	60 min.
	O.D.			
EA	1.170, 1.180	.890, .850	.790, .752	.541, .535
EA (EDTA)	.039, .030	.026, .024	.028, .031	.021, .031

ponent of C' reacts prior to C'1. even in the absence of cations. the further precaution of using purified rabbit antibody(8,9) may be necessary.

Summary. Commercial rabbit antisheep cell hemolysins contain rabbit C'1 which combines with the sheep erythrocyte-rabbit antibody complex. Heating at 56°C for one hour does not completely destroy the activity of the C'1. EDTA blocks the reaction of rabbit C'1 and can be used to elute rabbit C'1 combined with the erythrocyte-antibody complex.

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Received November 13, 1961. P.S.E.B.M., 1962, v109.

Effect of Diets and Hormones on Two Urea Cycle Enzymes.* (27215)

R. A. FREEDLAND AND C. H. SODIKOFF

University of California School of Veterinary Medicine, Agricultural Experiment Station, Davis, Calif.

Recently developed methods have made it possible to assay quantitatively all the enzymes in the urea cycle(1). It has further been shown that in most species, including the rat, the arginine synthetase system had the lowest activity of enzymes participating in the urea cycle(2). Arginine synthetase consists of 2 reactions, the conversion of citrulline and aspartate to argininosuccinate followed by the cleavage of this compound to arginine and fumarate. In all cases studied (2,3), the first of these reactions was shown to be the rate limiting reaction. Since there have been numerous reports(4,5,6) on the effect of high protein diets in raising liver arginase values and on the effect of adrenalectomy, which decreases protein catabolism, and causes a decrease in liver arginase and

* This work was supported in part by U.S.P.H.S. National Institute of Health Research Grant A-4732.