The Alteration of Blood Group Antigens by Carbodiimide (36316)

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Green (1) and Bove (2) have reported the reduction of the Rh antigen activity on human red cells treated with *p*-chloromercuribenzoic acid. They felt that the chemical action interfered with sulfhydryl groups of the antigen and that this identified a major Rh determinant. In other contributions Dodd *et al.* (3) and Rule and Boyd (4) presented evidence that neuraminic acid (sialic acid) forms a part of the terminal of the Rh antigen and suggested model formulas. Recent reviews on the chemical structure of the Rh antigen stress the lack of definitive knowledge on the subject (5). Race and Sanger (6) in their latest edition refuse to commit themselves on the chemistry of Rh antigen because of the meager existing evidence.

Our interest in this problem intensified when we found that ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (CDD) (Fig. 1) a polypeptide bonding reagent, would suppress the Rh antigen and several other blood group antigens when used in saline solutions. This paper reports the physical conditions used to elicit this effect and absorption experiments employed to indicate the red cell antigen alterations.

Materials. 1. Carbodiimide (CCD) ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride[1] is a white water soluble powder derived from urea. The usefulness of these compounds was first described by Sheehan and Hlavka (7), who employed them in various peptide syntheses. Apparently the activity of this group of compounds is centered about the unstable amino bonds which will readily combine with carboxyl groups. Therefore, caution is recommended when handling these substances because the exact toxicity is not known.

[1] Ott Chemical Company. Muskegon, MI 49445.

2. Blood group serums. Blood typing and Coombs' testing were performed using two commercial antibody sources (Dade and Ortho). Manufacturers recommended procedures were followed.

3. Blood samples were available from Intermountain Red Cross Blood Center and Holy Cross Hospital Blood Bank. The ACD-treated red blood cells were washed in saline (0.9%) three times and packed before use.

4. Red cells were exposed to the following mixture prepared within 15 min before use:

CDD (dissolve in saline)	12.0 mg
Saline (0.9%)	1.6 ml
Packed washed red cells	1.0 ml

This suspension was mixed by inverting several times and incubated 30 min at 37°. Every 5 min, the tubes were inverted during incubation. Finally, the cells were washed three times in saline before testing. By this method, only very slight hemolysis was noted (see Table I, detailing other concentrations).

5. Antihuman globulin testing (Coombs'). After treating the red cells with CDD, a routine anti-Rh test was repeated using 2 drops of anti-Rh (D) serum and a 2% suspension of the washed red cells. Centrifugation and reading invariably confirmed a negative test; and then a drop of saline was added to resuspend the cells and the tube was incubated 0.5 hr at 37°. As usual, three washes with saline followed; and then 1 drop of antihuman globulin was added as per manufacturer's recommendations. Finally, moderate centrifugation and reading from negative to 4+ completed the test.

Experimental Results. 1. Effect of CDD concentration on red blood cells. Generally speaking, the greater the concentration of

TABLE I. Effect of Concentration of CDD on Rh Typing of Washed Human Rh+ Cells (1 ml packed).

CDD (mg/ml of saline)	Anti-Rh reaction	Hemolysis 0–4+
6	++	0
7	+	0
8	±	0
9[a]	—	0
10	—	± (Trace)
20	—	± (Trace)
30	—	+
Control	++++	0

[a] A concentration of 9 mg of CDD/ml of saline or 12 mg/1.6 ml of saline was selected as the ideal concentration and volume to prepare red cells for testing.

CDD, the more hemolysis may be expected; but an optional concentration of minimal hemolysis in saline is obtainable so that cells will survive several days *in vitro*. Table I shows this parameter.

A concentration of 12 mg of CDD dissolved in 1.6 ml of saline was chosen as sufficient activity to treat 1 ml of packed, washed Rh positive cells. This has proven to be the correct level for suppression of Rh antigen, as well as several other antigens.

2. *Effect of temperature.* The activity of CDD upon red cells is dependent on proper temperature during incubation. Maximum effect is obtained at 37°, although some suppression of D activity is possible at room temperature. At 4°, very little suppression of Rh antigen occurs. Kell antigen suppression seems to depend upon 37°.

3. *Effect of incubation time.* At least 10 min incubation is necessary to inhibit Rh activity but the Kell antigen requires 30 min for complete inhibition. These cells, stored for 48 hr at 4° after the original treatment, gave the same negative results. We have extended this time to 96 hr storage without reversing the effect of CDD upon Rh positive cells.

4. *Anti-D adsorption by CDD-treated Rh+ red cells and untreated Rh+ red cells.* If the Rh antigen is truly nonfunctional after CDD treatment, then it should fail to adsorb anti-D in various dilutions. Table II shows this effect.

This table shows a spectacular difference in the ability to adsorb anti-Rh from diluted antiserum by CDD-treated O Rh+ cells as compared to normal O Rh+ cells. Normal cells adsorbed much of the anti-D antibody as expected, while CDD-treated O Rh+ cells behave in the same manner as Rh negative cells. Even after 48 hr storage, the CDD inhibited cells give the same reaction. We have not been able to reverse this reaction by heat (45°) or multiple saline washes.

5. *The effect of CDD on several blood group antigens of red cells* (Table III). The ABOH system is not affected by CDD. At present we have tested 10 cells of each of the following blood group systems as indicated. A more extensive survey is planned. It is noteworthy that the Rh system is clearly divided in that the C, c antigens are unaffected by CDD. The Tj[a] antigen, a composite of P antigens, is surprisingly persistent while P is depressed. It is to be emphasized that those cell antigens not suppressed by CDD give clear-cut reactions similar to a typing of untreated red cells. In some instances the reaction seems even sharper and hemolysis does not occur.

Since CDD suppresses the agglutination of Rh+ red cells we have extended the

TABLE II. Adsorption of Anti-Rh (D) Serum by Normal and CDD-Treated O Rh+ Red Cells.[a]

Dilution	Titer of unabsorbed anti-Rh serum	Titer after adsorption with: Normal Rh+ cells	Titer after adsorption with: CDD treated Rh+ cells
0	++++	++++	++++
1:5	++++	+	+++
1:10	++++	+	+++
1:20	+++	—	++++
1:40	+++	—	+++
1:80	++	—	++
1:160	+	—	++

[a] Each anti-Rh dilution tube was incubated and cells were washed for the standard Coombs' test. The inability of CDD-treated red cells to adsorb anti-Rh is indicated in the right hand column. ++++ = maximum agglutination; — = no agglutination.

TABLE III. Effect of CDD (12 mg/ml) on Packed RBC of Various Blood Group Systems.

Suppressed	Not suppressed
Rh: D,E,e	A_1 A_2 B H
Kell: K,k	C, c
Duffy: Fy^a	Tj^a, s
M,N,S,P	
Jk^a	Jk^b

procedure to include the indirect Coombs' test with antihuman globulin (AHG). Many Rh+ cells were tested and found negative for Rh antigen detection by antihuman globulin method after CDD. However, an occasional CDc/cDE cell would give a weak reaction, suggesting all antigen sites were not completely neutralized.

We did find that with the addition of glucose (200 mg/100 ml), or albumin (5%) to the CDD incubation diluent, the Rh typing was suppressed but that the antihuman globulin (AHG) produced its usual strong agglutinating reaction. This suggests several modes of action of CDD on the red cell surface.

Discussion. The finding that a carbodiimide compound has the ability to suppress the Rh (D) antigen may add one more stepping stone in our knowledge of the terminal antigen configuration. The probable deduction from this preliminary study adds strength to the theory of Dodd *et al.* (3) and Rule and Boyd (4) that sialic acid, with its prominent carboxyl group is a part of the Rh and other blood group antigen units of the red cell. In support of this concept, human serum, human albumin (5%), and sialic acid (1.0 to 5 mg/ml) were added to the CDD solution used to suppress the red cell antigens. Very little effect was noted from the protein solutions but sialic acid in 3–5 mg/ml prevented the action of CDD on the red cell surface. This is depicted graphically in Fig. 1.

This is probably a simplified concept because it is likely that other amino groups of proteins on the cell surface are involved. The indirect globulin test would perhaps attest to the complexity of the reaction. Indeed, it is not impossible that the carbodiimide initiates a polypeptide linkage to the Rh antigen.

We have injected CDD-treated red cells into rabbits intravenously and so far no toxicity has been observed. The antibody stimulation of these altered cells is to be reported in another communication. In addition, up to 150 mg of CDD has been injected into four rabbits with one fatal result while three were unaffected.

From these pilot-type studies of CDD effects on red cells it would seem logical to speculate that at least two terminal factors exist on the Rh antigens; one, a sialic acid-like structure which these studies show to neutralize CDD effect and another, a "globulin receptor" site which may still survive on "glucose protected" Rh+ cells even after CDD exposure.

With compounds like CDD, a whole new effort of antigen modifications seems possible and we intend to explore the route of Rh sensitization of animals and human volunteers with the hope that Rh antigen blocked cells could be used for transfusion. It is too early to predict this outcome.

Summary. Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (CDD) inhibits the expression of many blood group antigens of the human red cell. A notable exception is the ABO system. The alteration did not seem to be reversible by ordinary methods. Sialic acid blocks the effect of CDD

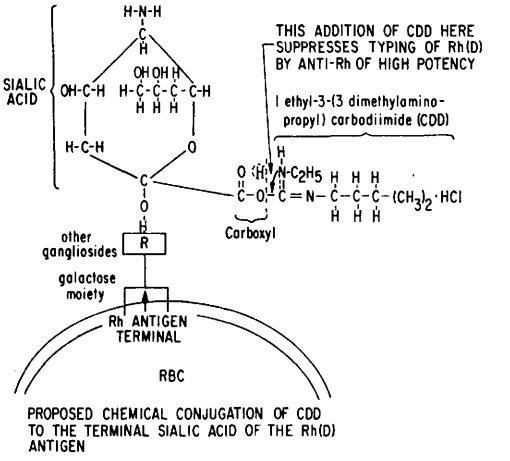

FIG. 1. A hypothetical diagram of carbodiimide binding to the terminal carboxyl group of the sialic acid portion of an Rh complex: This alteration seems to convert Rh+ cells to Rh—.

and may indicate the structure of the terminal chemical configuration.

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Received Oct. 25, 1971. P.S.E.B.M., 1972, Vol. 139.