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Studies of the effect of heat on RH positive blood cells

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STUDIES OF THE EFFECTS OF HEAT ON RH POSITIVE BLOOD CELLS

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INTRODUCTION

Levine and Stetson in 1939 (1) found an antibody in the serum of a woman who had just given birth to a stillborn infant. Previously she had been transfused with her husband's blood and had suffered a hemolytic reaction. This antibody was later found to be of the same specificity as the antibody produced by rabbits when injected with red cells of a Rhesus monkey.

Landsteiner and Wiener in 1940 (2) demonstrated that human blood would react with rabbit immune sera produced with blood of Rhesus monkeys and designated this as Rh property of human blood.

In 1941, Landsteiner and Weiner (3) recognized the importance of differentiating tests for Rh positive and Rh negative blood. By injecting cells from Rh positive and Rh negative individuals into guinea pigs,they found that the antisera produced was immunologically different.

With the correlation of the Rh factor to erythroblastosis fetalis by Levine and others (4), many investigators had started to work on the detailed study of the Rh system. A detailed

history of the Rh system can be found in the book Blood Groups in Man by R.R.Race and R.Sanger (5).

NATURE OF RH ANTIGEN

Calvin and others (6) prepared a 10% solution of red blood cell stroma and incubated this with serial dilutions of anti Rh (D) antibody. Following this they incubated the solution with strongly reactive Rh positive cells and noted inhibition of the agglutination of the red cells. They noted that heating the stroma to 56 degrees centigrade produced an inhibitory effect on the stroma antigen. They concluded then that the stroma of the erythrocyte had protein and lipoprotein moieties which were associated with Rh sensitivity.

Fisher (7) concluded that by determining the genetic frequencies of the Rh antigens there are three distinct pairs of elementary antigens. These antigens determine the Rh phenotype.

Carter (8) described the Rh hapten as a lipid, being insoluble in water and soluble in 95% alcohol, ether, chloroform and acetone, giving a positive Lieberman-Burchard Reaction (relating to cholesterol and unsaturated steroid). It gave a negative Biuret test (protein) and a positive Molish test (carbohydrates). The hapten contained 2% nitrogen

and 0.5% to 1.0% phosphates. This substance was antigenic when it was injected in conjunction with albumin into guinea pigs and had an inhibitory effect in a test tube.

Moskowitz and Dandlinger (9) identified the fraction of the stroma of the red blood cell which contains the Rh, A and B factors as elinin. They related that the Rh factor was either a protein or a simple molecule bound to the protein portion of elinin. The Rh factor was unstable under a variety of conditions which were favorable for protein denaturation such as heating elinin at 56 degrees centigrade for five minutes, adding ether and digesting the substance with trypsin. They were unable to reproduce Carter's work.

Boyd and others (10) used inhibition experiments using L-glucose, L-mannose, and D-gulose. They concluded that the D-receptor of Rh positive red blood cells contain one of the unnatural hexoses such as: L-glucose, L-mannose, D-gulose, or sugars of similar configuration. Anti C and anti E antigens were also inhibited. They also thought that the Rh antigens are at

least partly nucleotide in nature.

Dodd and others (11) used sialic acids for inhibition experiments. They stated that sialic acid occupied the terminal position in gangliosides and mucoprotein and contained a ketosidic linkage. They postulated that the terminal neuraminic acid bound to the ketosidic linkage conformed closely to determinant groups of Rh_o (D) antigen.

Hackel and others (12) by using inhibition experiments testing 60 chemical solutions, found that four solutions served to inhibit the Rh antigen-antibody reaction. These were cytidine sulfate, adenylic acid, uridylic acid and cytidylic acid which were derivitives of ribonucleic acids. They thought that the specificity of the Rh antigen is in part due to ribonucleic acids or ribonucleic acid like substances.

Race and Sanger (5) concluded that although no Rh substance had been successfully extracted thus far, inhibition experiments have revealed compounds similar to the antigen in such a way to give a clue to the chemical nature of the antigen. They postulated that if the Rh antigens

actually are polysaccharides, they cannot be direct products of DNA but are enzymatically different from DNA by one enzyme.

RH ANTIBODIES

Inhibitory Antibodies

Diamond and Abelson (14) concluded that there was an inhibitor substance within certain antisera preparations which interfered with the action of the agglutinin on the agglutinogen. They derived a rapid slide test to demonstrate the presence of anti Rh agglutinin by using higher concentrations (50%) of red blood cells in order to overcome the inhibition.

The same authors (15) later stated that the red cells can differentially absorb the inhibitory antibodies and the precipitating antibodies.

Weiner (16) thought that the blocking antibodies are of great clinical significance in the sensitized cells of erythroblastotic patients. He postulated that the blocking antibody was monovalent and the precipitating antibody was divalent.

Coombs (17) demonstrated that by using rabbit sera which had previously been sensitized to human blood cells, an anti human globulin could be extracted which would react with the sensitized cells. The incomplete agglutinins which were

present on the cells could then be demonstrated. He later (18) described a method of testing the sera of the mother for circulating blocking antibodies.

These tests are of use presently in hemolytic disease and diseases which have autosensitization as an essential factor.

Pickles (19) by using cholera filtrates and Morton and Pickles (20) by using trypsin have developed further tests for the demonstration of incomplete antibody.

Structure of Rh Precipitating Antibodies

Franklin and Fudenberg (21) described the antigenic heterogenicity of Rh antibodies. They relate that there are two antigenicly distinct classes of light chains which are common to all immune globulin. These are classified into 2 major groups: Type I and Type II. Anti Rh antibodies fall predominantly into the major antigenic Type I gamma globulins. Some are seen in Type II. There are reportedly several anti Rh antibodies which are in Type I and Type II groups.

Rawson (22) related that all blood group

antibodies are found in the three immune globulin fractions. These are $gamma_{ss}$, $gamma_{1A}$, and $gamma_{1M}$. Anti D antibody is present in the $gamma_{ss}$ fraction. The $gamma_{ss}$ fraction is subdivided into S division and F division. The anti D activity originates in the S portion,and the incomplete antibody activity originates from the F portion.

DYNAMICS OF ANTIGEN ANTIBODY REACTION

Rh System

Evans and others (23) stated that the antigen-antibody reaction in the Rh system was reversible and varied with the different antibodies in the system. They resolved that the Rh antigen-antibody reaction conformed to the mass action equilibrium relationship. They found that within the zone of antigen excess, the concentrations of antibody in the sensitizing medium determined the amount of antibody adsorbed to the cell. They stated that there was an inverse relationship between the number of univalent antibody molecules adherent to the cell surface and the number of molecules required to produce agglutination.

Antigen-Antibody Systems in General

Cruickshank (24) concluded that since antigen-antibody reactions do not form fast enough and are not reversible enough, covalent bands play no part in the antigen-antibody reaction.

He brought forth the following possibilities for the intermolecular forces acting between the antigens and the antibodies:

1. Coulomb Forces. These exist between $-COO^-$ groups on the dicarboxylic and amino acids, and $-NH_3$ groups on lysine as on protein antigens reacting on antibody globulins.

2. Van der Wall's Forces. These exist between any two molecules and depends upon instantaneous dipole movements created by movements on the center of charge of the rapidly moving electrons in relationship to charged molecules.

3. Hydrogen Bonds. These result from attraction of a hydrogen atom attached to one electronegative atom for an unshared electron pair of another electronegative atom.

He discussed the building up of aggregates forming a lattice structure in three dimensions in which bivalent antibody molecules link up with adjacent antigen-antibody complexes. He related that in antigen excess, the determinant groups would already be occupied by antibody; thus, the aggregates would be smaller.

Weinbach (25) related that the antigen-antibody reaction can dissociate under conditions of increasing ionic strength and extreme pH values.

He stated that the specificity of the antigen-antibody reaction groups is due to the reactive group of the antibody being complementary to the antigenic binding site. The antigen possessed the electron donor and accepted an antibody of characteristic strength. Each antibody was reported to have 2 identical reactive groups. He stated that the occurence of an antigen-antibody complex was decided by the formation of ions in the binding groups of the antigen and antibody.

Beiser and Tanenbaum (26) described the binding sites of the antibody. This site contains an aromatic glycone, an azo group and a galacto pyranosyl group.

Koshland (27) likened the active sites of enzymes to those of antibodies in 3 respects: First, there was evidence that residues far removed from each other in sequence, play a vital role in the active site. Second, the size of the active sites were similar. Third, the active sites were flexible in both the enzymes and the antibodies.

Masouredes (28) and others discussed antibody excess in Rh system in terms of decreasing the

electrostatic barrier resulting in less ionic groups on the red blood cells stroma after the cells had been modified with papain.

EFFECTS OF HEATING RED BLOOD CELLS

Inhibition of Rh Antigen-Antibody Reaction

Lubinski and Portnuff (29) demonstrated that Rh positive red blood cells when heated at 56 degrees centigrade failed to agglutinate with homologous anti Rh serum. They concluded that the Rh receptor was probably more superficial than those on Type A and Type B red cells, since heating to 56 degrees centigrade did not inhibit the agglutination of these cells when tested with anti A and anti B sera.

Hubinont (30) repeated the work of Lubenski noting similar results. By using inhibition studies, he demonstrated a Rh substance in the supernatant saline. He concluded that heating at 56 degrees centigrade splits off a substance from the surface of the cell which diffuses into the medium. He thought this substance was the Rh receptor.

Production of Anti-D Sera from Guinea Pigs

Murray and Clark repeated the experiments of Lubinski and Portnuff and found that anti-D sera was produced in guinea pigs by intraperitoneal injections of the saline supernatant from the Rh positive and Rh negative heated cells(31). This

anti sera was potent for two years. They explained that a single substance forms basic material for all Rh antigens and the specificity of the Rh antigens is based on the side chain groups . Anti c titers were also produced however the levels were not as high as with anti D sera.

Ponder and Ponder (32) demonstrated that by heating red blood cells at various temperatures from 50 degrees centigrade to 56 degrees centigrade the red cell surface appears to become fragmented and there is the development of myelin forms. At higher temperatures they produced hemolysis. It was thought that the substances in the surrounding media are products of fragmentation phenomenon and that the changes in the red cell surface accompany this. They found that upon heating the cells the serologic reactivity becomes progressively weaker as the temperature increases and the agglutination was lost at 56 degress centigrade. Incomplete antibodies as shown by indirect Coombs reaction were inhibited on the red cell surface but did not become modified to give a positive direct Coombs reaction.

Murray (33) demonstrated that guinea pigs have an anti D sera which will react with Rh positive cells; however, the sera differs from human anti D sera. It is thought that guinea pig immune sera contains specific anti monkey antibodies as well as anti D. This sera contains antibodies to another human characteristic shared with monkey which reacts very weakly in saline and more strongly by indirect Coombs tests and with albumin techniques.

Of significance is that before injection of saline supernatants into guinea pigs the levels of anti D sera should be determined in order to note any change in antibody concentrations following testing with supernatants.

Differentiation of Anti D Sera from Guinea Pigs as Compared to Human Anti D Sera

Levine (34) in following the work of Murray noted a D-like antibody produced in the guinea pigs' serum which differed from human anti D in respect to the following: First, Rh positive cells which were blocked with powerful commercial anti D serum, still agglutinated when exposed to the anti D-like

substance produced by the guinea pigs. Second, the D-like antibodies produced by injecting any material containing the D-like antigen, when exposed to Rh positive, Rh negative or Rhesus red blood cells will agglutinate Rh positive cells. However Rh negative red cells possessing the D-like antigen when exposed to human anti D sera will not agglutinate.

Levine and others (35) heated red cells at 50 degrees centigrade and used high speed centrifugation to yield the myelin fraction described by Ponder and Ponder (32) from the fragmented heated cells. This was used to immunize guinea pigs. The results were strongest with Rh negative cells. The guinea pig sera would agglutinate Rh positive cells already coated with blocking antibodies; whereas, saline anti D would not agglutinate the red cells. In gel diffusion tests the D-like substance failed to yield precipitin bands. The results demonstrated that the guinea pig sera has specificity only superficially simulating human anti D. The authors concluded that the guinea pig anti D sera acts on different site that human

anti D sera. They also concluded that guinea pig reagents seem to define an agglutinable property which differs from human antigenic determinant but seems to be present in Rh positive, Rh negative and Rhesus cells and feel that "Rhesus factor" seems to be a misnomer. They speculate that the use of guinea pig anti D substance can be used to differentiate Rh type of cord blood more accurately than normal anti D sera used presently in standard laboratory techniques.

Levine and Celano (36) applied the elution techniques and antigenicity to guinea pig sera to blood of a patient with ---/--- genotype. They failed to elicit a rise in antibody titer and concluded that the D-like antigen is associated with all human bloods bearing any one or more antigen determinants of Rh-Hr system.

In our laboratories, Hobel (37) attempted to increase anti D titers by injecting heated Rh stroma into rabbits. The results showed an elevated anti D titer. Various solutions to inhibit Rh antigen-antibody reaction were injected into these rabbits; the results were inconclusive.

EXPERIMENTAL WORK

Repeating the Work of Hubinont and Others:

Experiment I

The work of Hubinont was repeated and expanded. The technique was as follows:

Heating Red Cells

A sample of blood group O Rh positive whole blood was collected from the hospital laboratory and centrifuged, yielding at least one cubic centimeter of packed cells. A one cubic centimeter aliquot of these blood group O Rh positive cells was washed three times with 0.85% saline and suspended with an equal volume of 0.85% saline which was adjusted to pH of 7.2. This suspension was then heated for 15-20 minutes in a water bath at a temperature of 56 degrees centigrade. The suspension was centrifuged at 2000 RPM for 5 minutes and the supernatant saline solution was separated and collected. The heated cells were washed again with 0.85% saline for three times and a 2% solution of heated red cells was prepared using saline as a diluent. Also 2% solutions of unheated red cells of blood groups O Rh negative and group

O Rh positive cells and heated group O Rh negative cells were used as controls.

Preparation of Serial Dilutions of Anti Sera

Serial dilutions of Dade anti D saline anti sera were made using 0.85% saline and the anti D anti sera. The dilutions were made as follows: 0.05 cc. saline to 0.05 cc. anti sera and continued in 1:2 dilutions. The dilutions were carried out to the tenth tube yielding a final dilution of 1:512.

Procedure of Adding Cells to Anti Sera

After preparing multiple dilutions of anti sera, 0.05 cc. of 2% red blood cell suspensions were added to a mixture of 0.05cc. anti D sera and 0.05cc. of 0.85% saline. These solutions were then incubated in a water bath at 37 degrees centigrade for 30 minutes.

Rating Degree of Agglutination

After the incubation period, the test tubes were centrifuged at 2000 RPM for 5 minutes and the centrifugate was inspected for gross and microscopic agglutination. The microscopic agglutination was read according to the Race Scale(38).

See Table I.

Table I

Agglutination clearly visible to naked eye	10
Very large agglutinates seen microscopically	8
Large agglutinates seen microscopically	5
Small agglutinates seen microscopically	3
The smallest definite agglutinates	2
No agglutination and cells evenly distributed	0

The scores are added for each experimental group, and the results of each groups are compared against each other.

For results of Experiment I, see Table II.

Table II

Experiment I:

Identification for Controls and Test Solutions

C_1 --- Saline control using 2% type O Rh negative cells, saline, Dade anti D sera

C_2 --- Saline control using 2% type O Rh positive cell, saline and Dade anti D sera

T_1 --- Test solution of heated type O Rh positive cells, saline, and Dade anti D sera

Results

	C_1	C_2	T_1
1/1	0	10	3
1/2	0	10	2
1/4	0	10	0
1/8	0	10	0
1/16	0	3	0
1/32	0	0	0
Total	0	43	5

The Testing of the Antigenicity of the Supernatant Fluid: Experiment II

In order to test whether the antigen which was thought to be freed into the solution by heating was active in the solution and would again combine with the red cells which were heated, the following study was done:

2% suspension of blood group O Rh positive cells in saline, heated blood group O Rh positive red cells in saline and heated blood group O Rh positive red cells in supernatant fluid were again tested against serial dilutions of Dade anti D sera. After incubating again for 30 minutes at 37 degrees centigrade, the tubes were centrifuged at 2000 RPM for 5 minutes, and the degree of agglutination was read according to the Race Scale. Coomb's tests were run on the first dilution of anti sera to show no agglutination macroscopically and microscopically.

Results of this experiment are seen in TableIII.

Table III

Experiment II:

Identification for Controls and Test Solutions

C_1 --- Saline control using 2% suspension type O Rh positive red cells, saline, Dade anti D sera

H_1 --- 2% suspension heated type O Rh positive, red cells, saline, anti D sera

H_2 --- 2% suspension heated type O Rh positive red cells in supernatant fluid, saline and anti D sera

Results

	C_1	H_1	H_2
1/1	10	2	3
1/2	10	0	2
1/4	10	0	0
1/8	5	0	0
1/16	2	0	0
1/32	2	0	0
1/64	0	0	0
Total	35	2	5

Coombs Test	Dilution 1/64 on C_1	10
	Dilution 1/2 on H_1	3
	Dilution 1/4 on H_2	8

Determination of the Exact Concentration of Antigen in the Supernatant Fluid: Experiment III

A 50% suspension of blood group O Rh positive red blood cells was heated at 56 degrees centigrade for 15 minutes, and the supernatant was removed. The remaining intact red blood cells were then washed and a 2% saline suspension of these cells was prepared. This 2% suspension was then incubated with various concentrations of anti D sera according to the saline tube method already described (37 degrees centigrade for 30 minutes) and read for agglutination by the Race method. Coomb's sera was then used to read the "0" readings for any anti D sera possibly remaining on the cells. The test reaction was performed as follows:

First, the supernatant from the heated 50% blood group O Rh positive red blood cell solutions was incubated with various dilutions of anti D anti sera at 37 degrees centigrade for 15 minutes. Second, this mixture was then incubated at 37 degrees centigrade for 30 minutes with a 2% blood group O Rh positive red cell

suspension. Third, if there were no agglutination in these tubes, a Coombs test was run.

The results of this experiment are seen in Table IV.

Table IV

Experiment III:

Identification for Controls and Test Solutions

C_1 --- Albumin, saline anti D sera and 2% suspension type O Rh positive red cell

C_2 --- Saline anti D sera, 2% suspension of type O Rh positive red cells

H_1 --- Saline anti D sera, 2% suspension of heated washed red cells in saline

H_2 --- Saline anti D sera, 2% suspension of heated washed red cells in supernatant fluid

H_3 --- Saline anti D sera, 2% suspension of type O Rh positive red cells in supernatant fluid

Results

	C_1	C_2	H_1	H_2	H_3
1/1	10	10	2	0	10
1/2	10	10	2	0	10
1/4	10	10	0	0	10
1/8	10	10	0	0	10
1/16	2	10	0	0	10
1/32	5	3	0	0	5
1/64	3	2	0	0	2
1/128	3	0	0	0	0
1/256	2	0	0	0	0
1/512	0	0	0	0	0
Total	55	55	4	0	57

Coombs Test: Dilution 1/4 on H_1 3

Dilution 1/1 on H_2 10

Dilution 1/128 on H_3 10

The Effect of 10% Glucosamine on the Supernatant Antigen: Experiment IV

This study was performed to test the inhibitory effect of 10% glucosamine on the Rh antigen-antibody reaction as described by Pearse and Hobel(13). Heated red cells of blood group O Rh positive and O Rh negative were prepared as described previously by heating them at 56 degrees centigrade for 30 minutes, and the supernatant fluid was retained. This fluid was used to prepare a 2% suspension of heated cells of blood group O Rh positive. Then, 2% suspensions of blood group O Rh positive and blood group O Rh negative red cells were prepared using 0.85% saline as diluent.

Serial dilutions of saline anti D sera were prepared as previously described. These were mixed with the various cell suspensions as seen in Table V.

A buffered solution of 10% glucosamine was added to test solutions H_5 and incubated for 30 minutes at 37 degrees centigrade. After the incubation period fresh red cells of blood group O Rh positive were added to the mixture.

All the tubes were incubated at 37 degrees centigrade for 30 minutes with the exception of test solutions H_3, which were incubated at 56 degrees centigrade for 30 minutes in order to destroy the complement.

All the test solutions were centrifuged at 2000 RPM for 3 minutes and scored acording to the Race scale. Coombs tests were performed on the first negative results in each test solution.

Results of this experiment are seen in Table V.

Table V

Experiment IV:

Identification for Controls and Test Solutions

C_1 --- 2% Type O Rh negative cell suspension in saline, anti D sera and saline

C_2 --- 2% Type O Rh positive cell suspension in saline, anti D sera and saline

C_H --- 2% Heated Type O Rh negative cell suspension in saline, anti D sera and saline

H_1 --- 2% Heated Type O Rh positive cell suspension in saline, anti D sera and saline

H_2 --- 2% Heated Type O Rh positive cell suspension in supernatant, anti D sera and saline

H_3 --- 2% Heated Type O Rh positive cell suspension in supernatant, anti D sera and saline -- incubated at 56 degrees centigrade

H_4 --- 2% Type O Rh positive cell suspension in saline, added after supernatant, anti D sera and saline were incubated at 37 degrees centigrade for 15 minutes

H_5 --- 10% Glucosamine, anti sera, supernatant incubated at 37 degrees centigrade for 30 minutes and then 2% type O Rh positive cell suspension in saline was added

Note:
Table V continued on next page.

Table V (continued)

Results

	C_1	C_2	C_H	H_1	H_2	H_3	H_4	H_5
1/1	0	10	0	3	0	0	10	10
1/2	0	10	0	2	0	0	10	10
1/4	0	10	0	2	0	0	10	8
1/8	0	10	0	0	0	0	10	0
1/16	0	10	0	0	0	0	10	0
1/32	0	10	0	0	0	0	10	0
1/64	0	8	0	0	0	0	5	0
1/128	0	5	0	0	0	0	3	0
1/356	0	3	0	0	0	0	2	0
1/512	0	2	0	0	0	0	0	0
Total	0	78	0	7	0	0	70	28

Coombs Test:	Dilution 1/1 on C_H	10
	Dilution 1/8 on H_1	10
	Dilution 1/1 on H_2	10
	Dilution 1/1 on H_3	0
	Dilution 1/512 on H_4	10
	Dilution 1/8 on H_5	10

DISCUSSION

Experiment I was a repeat of Lubinski and Portnuff's (29) work concerning heating of Rh positive red blood cells. It showed that heating the cells resulted in a loss of antigenicity of the cell. The antigen which was on the surface of the cell was apparently altered on the cell wall or released from the cell as described by Hubinont (30).

In Experiment II the antigen apparently did not recombine with the heated red cells after adding the red cells to the supernatant solution. It was noted that the cells had changed in color and there was ghosting of the cells and marked change of cellular structure. The quantity of antigen in the solution was not known and the concept of antibody excess may be the cause for this failure to agglutinate the red cells. The Coombs reaction showed some increase in values; therefore, there was probably some coating of the cells with the antibody.

Experiment III again demonstrated that the heated red cells were not reactive with the antibody.

It appears that the supernatant antigen does not recombine with the heated cells. Also it appears that the antigen has no inhibitory effect against a fresh red cell suspension.

The explanation of the positive Coombs results in the heated cell suspension , fresh cell suspension in supernatant fluids, and the heated cells in saline is most likely the fact that the incomplete antibody is present in the anti D sera. The anti D sera was the common ingredient in the above test solutions.

Experiment IV heating the red cells at 56 degrees centigrade in H_3 showed a reduction in Coombs values which showed that the incomplete antibody was altered or destroyed yielding a negative Coombs reaction. In H_4 the purpose was to have the antigen in the supernatant fluid, if present, react with anti D sera. The blood type O Rh positive cells served as an indicator. A lack of agglutination indicated a positive antigen-antibody reaction with the supernatant and anti D sera. There results show that if the antigen were in the supernatant, it was weakly reactive

with the antibody or not competing at all with the strong antigen on the type O Rh positive cells.

Tubes H_5 showed an inhibitory effect by glucosamine when it was added to the solution, however, the inhibition was not complete as evidenced by a score of 28. Possible reasons are the following:

First, there was an increased dilution of the system by the addition of supernatant. Second, there was actual interference with the supernatant "antigen." Third, the solution of glucosamine was weakened resulting in an ineffective concentration of glucosamine.

Of importance is the possibility that the cells are altered to a point that antigen excess and antibody excess are major complicating factors. The amount of antigen released into the fluid may be extremely low and any amount of antibody added may not react with the small amount of antigen.

SUMMARY

This paper attempted to cover the problem introduced by Lubinski and Portnuff (29) at which time they noted that upon heating Rh positive red blood cells the ability of the red cells to react with anti D sera was lost. In studying the literature covering the subject of heating red cells, it was found that there is antigenic activity in the supernatant fluid as evidenced by increasing anti D titers in guinea pigs and rabbits. The actual physical change in the cell wall was described leading to extrusion of cellular particles called myelin. It was thought that this caused the antigenic activity of the Rh antigen. Our own experiments have shown a failure of Rh positive cells to agglutinate after heating. Efforts to demonstrate an antigen within the supernatant have not been fruitful. Inhibitory experiments using 10% glucosamine did show decrease in agglutination of the red cells in Rh antigen-antibody system completely.

An attempt was made to relate recent ideas concerning Rh antigen structure and composition

and Rh antibody composition. The factors influencing antigen-antibody reaction also were covered implicating ionic strength of the solution and pH of the solution as being of major importance. Mechanism of Rh antigen-antibody reactions related the importance of antigen excess and antibody excess influencing the mass action combination of the antigen with the antibody. The reaction was thought to be not one of strong chemical interaction but that of weak intermolecular forces readily influenced by concentrations of the antigen and antibody.

This paper present many possibilities for further studies.

Studies are presently being undertaken in our laboratories concerning the mechanism of Rh inhibition using various sugar compounds.

We also are continuing the heating experiments in hope to demonstrate antigenic activity in the supernatant fluid by using glucosamine as an inhibitor substance. The antigenic substances in the supernatant fluid will be further explored.

CONCLUSION

1. The history of the Rh system was covered in brief,and the physical chemical properties of Rh antigen and antibody were discussed.
2. Factors governing the Rh antigen-antibody reaction were reviewed, and possible mechanisms were introduced.
3. Literature covering effects of heating Rh positive red blood cells was reviewed, and experimental data covering preliminary research on this subject was described.
4. There is opportunity for further research in the field of heating red cells, which may be of help in further identification of the Rh antigen.

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