STUDIES ON THE EFFECT OF GAMMA GLOBULIN ON COOMBS REAGENT TO NATURAL AND TO ACQUIRED ISOHAEMAGGLUTININS OF THE GROUP A SYSTEM

A Thesis

Submitted to

the Faculty of Graduate Studies and Research

University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

THE UNIVERSITY OF MANITOBA LIBRARY

by

F. C. C. Wong, B.Sc.

September 1959

ACKNOWLEDGMENT

The author wishes to express his gratitude to Dr. Rona Hawirko of the Department of Microbiology, for the advice and assistance accorded him in the course of the investigation.

ABSTRACT

The present investigation was undertaken to elucidate the difference between the natural occurring anti-A isoagglutinins and the immune anti-A isoagglutinins. Four antisera were prepared; Coombs serum to purified human gamma globulin AHG, Coombs serum to eluted natural anti-A AHG "A" (Halvorson), Coombs serum to eluted immune anti-A AHG "A" (Penner), and Coombs serum to eluted cord anti-A AHG "A" (Boadway). Antiglobulin tests were performed with the prepared Coombs sera on both the natural occurring and immune forms of anti-A isoagglutinins. Antiglobulin test on both forms of anti-A were also performed with Coombs serum AHG "A" (Halvorson) after the addition of gamma globulin some of the gamma globulin treated antisera were further added with AHG. Micro-estimations of the nitrogen contents of the precipitin to gamma globulin were performed on all the prepared Coombs sera.

The antiglobulin tests showed that the natural occurring anti-A was inactive to AHG but active to all other Coombs sera, and that approximately eight times more anti-A isoagglutinins were required for a positive antiglobulin tests with Coombs sera to immune anti-A than that required with the Coombs serum to natural anti-A. Eluted immune anti-A from Penner serum and from Boadway cord serum were active to all the Coombs sera including AHG. For a positive antiglobulin test, the amount of immune isoagglutinin required

was about the same with each Coombs serum. The absorbed cord serum in place of eluate was found to be inactive to AHG. Approximately 6.25 γ of gamma globulin were able to inhibit the agglutinating ability of 0.2 ml. of AHG "A" (Halverson) when tested with natural anti-A isoagglutinins whereas 2.34 γ were required for the same amount of AHG "A" (Halverson) when tested with immune isoagglutinin. The addition of 0.1 ml. of AHG 1/10 diluted to the neutralized Coombs serum reactivated its agglutinating ability in all cases. The milligram nitrogen contents of the precipitin to gamma globulin of the Coombs sera per unit gamma globulin at maximal precipitation were as follows: 1.46 for AHG; 3.52 for AHG "A" (Halverson); 0.965 for AHG "A" (Fenner); and 8.92 for AHG "A" (Rodney).

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
HISTORICAL	2
MATERIALS AND METHODS	12
Preparation of Coombs Reagents	13
Treatment of Antiglobulin Sera	14
Antiglobulin Test	14
"Neutralization" with Human γ globulin	15
Addition of Anti-human γ globulin	15
Quantitative Micro-estimation of Antibody to γ globulin in Coombs Reagents	15
Graphing of Precipitin Nitrogen	18
RESULTS	20
DISCUSSION	48
CONCLUSION	52
REFERENCES	53

LIST OF TABLES AND FIGURES

			PAGE
Table	1.	Cross Titration of eluted Anti-A on A_1 Cells with Antiglobulin sera	24
	1A	Eluted anti-A Halvorsen	24
	1B	Eluted anti-A Penner	25
	1C	Eluted anti-A Boadway	26
Table	2.	Reaction of Eluted Anti-A, Diluted 1:20, coated on A_1 Cells with Coombs AHG "A" Treated with Human γ globulin	27
	2A	Eluted anti-A Halvorson	27
	2B	Eluted anti-A Wong	28
	2C	Eluted anti-A Turbovsky	29
	2D	Eluted anti-A Baydack	30
	2E	Absorbed Boadway cord serum	31
Table 4.		Qualitative Precipitin Test on Coombs sera	38
Table	5.	Micro-estimation of the nitrogen content of Precipitin	39
	5A	Precipitin to anti-human γ globulin	39
	5B	Precipitin to anti-immune anti-A	40
	5C	Precipitin to anti-natural anti-A	41
	5D	Precipitin to anti-placenta cord anti-A	42
Figure	1.	Micro-estimation of the nitrogen content of the precipitates formed by the Coombs sera AHG with varying amount of γ globulin	43

PAGE

Figure 2. Micro-estimation of the nitrogen content of the precipitates formed by the Coombs sera AHG "A" (Penner) with varying amount of γ globulin 44

Figure 3. Micro-estimation of the nitrogen content of the precipitates formed by the Coombs sera AHG "A" (Halvorson) with varying amount of γ globulin 45

Figure 4. Micro-estimation of the nitrogen content of the precipitates formed by AHG "A" (Boadway) with varying amount of γ globulin 46

Figure 5. Micro-estimation of the nitrogen content of the precipitates formed by all the prepared Coombs sera with varying amount of γ globulin 47

INTRODUCTION

It is well known that antibodies to the A and B antigens in the blood group system occur in reciprocal relationship to the antigens; that is, they are distributed so that antibody is lacking in sera of an individual whose red cells contain the corresponding antigen. The concept that the isoagglutinins anti-A and anti-B are inherited by a set of genes completely linked to those determining the inheritance of the antigens was introduced by Furuhata (16) in 1927. Furthermore, since A and B antigens are present in most body tissues and, according to Race and Sanger (38), in the body fluids of approximately 80% of the world population, isoimmunization by ABO incompatible pregnancy is not infrequent. The antibodies thus produced have been variously termed "blocking", "incomplete", "univalent" and "immune". For the purpose of uniformity the term "immune" will used in this paper.

To differentiate between the natural and the immune antibodies, a number of criteria have been advocated by various workers notably Koopmaker and van Loghem (39) who have listed eight tests by which the natural and the immune anti-A and anti-B can be distinguished. The present study was undertaken to elucidate the difference, if any, between the natural anti-A and the isoimmune anti-A.

One of the means by which immune antibodies may be demonstrated is the antiglobulin test. The basis of

this test is as follows: when cells, suspended in saline, are exposed to the action of an immune antibody they are not agglutinated but they nevertheless absorb antibody to their surface. Antibodies are globulins and therefore red cells which have absorbed antibody can be regarded as coated with globulin. The globulin on the cell surface can be detected by exposing the cells to an antiglobulin serum which causes rapid agglutination of the cells. The principle of the antiglobulin test was demonstrated as early as 1908 by Moreschi (36), but the test did not come into general use intil 1945 when Coombs, Mourant and Race showed its application in detecting immune Rh antibodies (16). The antiglobulin test has since been also known as the "Coombs' test" and the rabbit antiglobulin serum has also been referred to as "Coombs reagent".

This study deals with firstly a qualitative evaluation of the specificity and reactivities of Coombs reagents prepared by immunizing rabbits with eluted natural anti-A and isoimmune anti-A, secondly the reaction of these antibodies to human gamma globulin and to Coombs reagents to human gamma globulin, and thirdly, a quantitative comparative study of the nitrogen content of the precipitates formed by gamma globulin with the prepared Coombs reagents by means of micro-Kjeldahl analysis.

HISTORICAL

The substances which characterize the blood groups are found on the surface of red cells. They are all antigenic and may stimulate the formation of antibodies. The principal way in which the blood group antibody can be recognized *in vitro* is by its ability to cause agglutination of the red cells having the corresponding antigen. In the early work on these antibodies the agglutination reaction was always carried out by mixing the serum containing the antibody with red cells suspended in physiological saline. When the antigen was present agglutination occurred, and by this means the ABO, MN, P, and Rh blood groups were discovered (27 to 30). With the discovery of the Rh blood group came the understanding of erythroblastosis foetalis, a haemolytic disease of the newborn, and the cause of many transfusion reactions. It was through the work on these clinical problems that much valuable knowledge concerning the diversity of reactions of the Rh antibodies was found. The discovery of immune forms of antibody in the Rh system initiated a search for the similar form in other blood-group systems. Many interesting findings, especially in the ABO system, have since been reported.

Haemolytic disease of the newborn due to incompatibility between ABO blood groups of mother and foetus has been reported to occur about once in every 2000 births (32). In 1905 Dienst (14) first demonstrated that foetal A or B

antigenic material may enter the maternal circulation to immunize women who lacked these antigens. Evidence for the aetiological role of anti-A and anti-B antibodies was presented in 1949 by Boorman, Dodd and Trinick (2), and by Mollison and Cutbrush (33) independently. The authors carried out red cell survival studies by a modified Ashby technique and demonstrated the shortened survival time of normal A erythrocytes transfused to affected group A infants.

Difference between Immune and Natural Anti-A

Until comparatively recently little stress has been laid upon qualitative differences between the immune and the natural forms of anti-A. The view held was that any strong anti-A serum would haemolyse A cells in the presence of complement. In 1941 Wiener (43) noted that following incompatible transfusions anti-A agglutinins were produced which showed a greater tolerance to change in temperature which he suggested to be a useful index of the formation of immune antibody. His observation implied that qualitative differences may exist between immune and natural occurring forms.

Diamond and Abelson (14) reported that a heavy suspension of Rh positive cells suspended in their own serum in the presence of immune anti-Rh produced rapid agglutination. In the same year, Boorman, Dodd and Morgan (4) observed that when anti-A sera were tested in halving dilutions against A cells, the reactivity in most cases was

enhanced by diluting with serum instead of saline. The effect was observed only when testing sera from individuals who had recently received an A stimulus and was not noted in sera containing natural occurring anti-A.

Meanwhile an immune form of Rh antibody had been described by Race (37) and by Weiner in 1944 (42) both of whom demonstrated this form to be more heat-stable than ordinary agglutinins. Two years later Boorman and Dodd (4) demonstrated a similar form of antibody in two immune anti-A sera. They showed that heating the sera at 75°C for 20 minutes destroyed the power of the sera to agglutinate cells suspended in saline solution. However, the sera so heated blocked the reaction between A cells and naturally occurring anti-A. Moreover, the heated sera agglutinated A cells upon the addition of anti-human serum.

Further characteristics of immune anti-A sera were described by Witebsky (44). He showed that, if certain anti-A sera were treated with sufficient AB substance, an extract containing A and B antigens, so that they would no longer agglutinate A cells suspended in saline, agglutination could be achieved if the suspension were made in serum. Such treatment of the sera with an appropriate dose of AB substance, which neutralized the saline agglutinins, was described as partial neutralization. Witebsky also showed that immune sera fixed complement in the presence of soluble A substance.

Haemolytic reactions caused by transfusion of group O blood to group A people were reported by Erwin and Young and by Erwin et al. (15). These investigators extended Witebsky's observations by showing that partially neutralized immune anti-A sera would agglutinate cells upon the addition of an anti-globulin serum. It was also noted that sera of twelve of a hundred random group O donors displayed immune characteristics.

Grumbach and Gasser (10) found that erythrocytes from infants of ABO incompatible pregnancy displayed an increased osmotic fragility. However, these results are difficult to interpret since neither details of method nor the normal range of value for newborn were presented. Increased osmotic fragility was also reported by Robinson et al. (40). A detailed study was carried out by Crawford et al. (8), who found that the median corpuscular fragility was above normal limits in ten of eleven cases and that in all eleven cases the fragility curves, plotted with per cent lysis of erythrocytes against per cent NaCl, had an abnormal shape. In comparing erythrocytes from infants sensitized by immune anti-Rh with those sensitized by immune anti-A, Crawford showed that changes in osmotic fragility in cases due to ABO incompatibility may persist for one to two weeks after birth longer than the Rh system but were less pronounced.

Coombs Reagent

The Principle of the antiglobulin test was introduced by Moreschi in 1908 (36) who demonstrated that red cells which had been exposed to various agglutinating sera in an amount insufficient to cause agglutination could be agglutinated by the addition of an anti-protein serum specific for the protein of the agglutinating sera but not for the cells tested. He further demonstrated that the same phenomenon applied to bacterial agglutination, and that this indirect method was far more sensitive than direct agglutination methods. Moreschi presumably did not realize that he was actually detecting the simultaneous presence of "incomplete" antibodies rather than true agglutinins.

Independently, Coombs, Mourant and Race in 1945 (7) suggested the same principle as a highly sensitive means of demonstrating incomplete anti-Rh antibodies. Saline suspended human red cells incubated with human serum containing specific incomplete antibodies are not agglutinated but are sensitized with antibody protein; the sensitization may then be demonstrated by adding rabbit anti-human-gamma-globulin serum, which selectively agglutinates red cells sensitized with human antibody (human gamma-globulin). This test is referred to as the Coombs' test or the antiglobulin test and the rabbit antiserum is termed Coombs serum or Coombs reagent.

In serological diagnosis of haemolytic disease of the newborn, the indirect antiglobulin test is employed to demonstrate the presence of immune antibodies in the maternal serum and the direct antiglobulin test is performed on the erythrocytes of the infant to detect sensitization. The test has been proven to be a reliable tool in diagnosis of Rh incompatibility. However, in haemolytic disease due to ABO incompatibility, the value of the antiglobulin test is yet uncertain.

Boorman and associates (2) found the direct antiglobulin test to be positive on the fourth day after birth in one severe case of haemolytic disease of the newborn due to anti-A, though the test was negative in another case examined on the tenth day after birth. Robinson et al. (40) reported that the direct antiglobulin test was positive, though not pronouncedly, in seven of eleven cases and negative in the remaining four. The weakness of the reactions appear to be due to some quality of the antigen rather than to the lack of antibody since, in two instances in which the direct antiglobulin tests were negative, the sera of the same infants markedly sensitized adult A_1 cells to an antiglobulin serum. In eleven cases described by Crawford, Cutbush and Mollison (8), all of which showed signs of a moderately severe haemolytic process, the direct antiglobulin test was negative in four cases and only weakly positive in the remaining seven. However, in 1955 Rosenfield (41) reported that if a modified antiglobulin test was used positive

results were not infrequent. His modification was essentially a macroscopic and microscopic examination, and aggregates of three or more red cells were considered to indicate a positive result.

Elution and Antiserum to Eluate

In 1925 Landsteiner and Miller (31) introduced a method for the recovery of antibodies from sensitized erythrocytes. The antibodies were eluted off the erythrocytes by heating in saline at 56°C. for five minutes and centrifuged leaving a supernatant which contained the eluted antibody.

The procedure has been used successfully to recover Rh antibodies from cells of erythroblastotic infants by Carter and Loughrey (5) and from cells of patients suffering from acquired haemolytic anemia by Crawley and Bouroncle (9). This method has been applied also to the separation and recovery of rare antibodies from antisera containing a mixture of antibodies by treatment with red cells of appropriate antigenic structure(25).

Immunochemical Analysis

In 1929 Heidelberger and associates (19) introduced a quantitative method, based on the rigid criteria of analytical chemistry, for the analysis of antigen-antibody systems by using a nitrogen-free, type specific polysaccharide of pneumococcous as antigen and a highly purified antibody solution. By varying the proportion of antibody solution

to antigen, a zonal phenomenon was recognized: firstly a zone of excess antibody, secondly a zone of equivalent in which neither antigen nor antibody could be detected in the supernatant fluid, and thirdly a zone of antigen excess which often proceeded into complete inhibition with increasing quantities of antigen. A quantitative theory of the precipitin reaction was formulated, based upon the combination of multivalent antigen with multivalent antibody in a series of competing bimolecular reactions (21). The behavior of an antiserum up to the region of antigen excess could be expressed by a simple linear equation. The validity of the theory has been reaffirmed recently by Gitlin and Edhoch (17), and by Goldberg and Campbell (18), on the basis of data obtained by light scattering. As a result of the theory, it became possible to predict a method for the preparation of analytically pure antibody, to achieve its isolation (20), and to furnish final proof that antibodies are actually modified globulins.

Extension of the quantitative method to precipitin reaction of protein-antiprotein systems initially required some means of distinguishing between antigen nitrogen and antibody nitrogen in order to determine the change in composition of the specific precipitate with varying proportions of antigen and antibody. For this purpose Heidelberger et al. (21) employed a dark red derivative of crystalline egg albumin synthesized by tetrazotizing benzidine and coupled on one side with R-salt and on the other

with egg albumin. With this coloured antigen, the nitrogen sources in the precipitates can easily be determined by a colorimetric analysis before micro-Kjeldahl. The results comfirmed the quantitative precipitin theory.

MATERIALS AND METHODS

Natural anti-A Sera

Sera were separated from the clotted blood of group B donors showing the following saline titres to A_1 cells: Halverson 1:256; Turbovsky 1:160; Wong 1:320; Haydack 1:320.

Immune anti-A serum

Immune anti-A serum was obtained from a group O donor (Mrs. Penner) showing a saline titre of 1:512 to A_1 cells after giving birth to a child with group A blood.

Placental cord anti-A Serum

Serum was separated from blood drawn from the placenta of Broadway after birth, and no detectable saline anti-A titre was obtained. However, 1:4 dilution of the serum yielded a titre of 1:80 to an indirect antiglobulin test.

Red Blood Cells

Citrated blood was made available through the courtesy of Miss Catherine Anderson of the Winnipeg Red Cross. Cells used for elution were A_2, and those for titrations and indirect Coombs' tests were A_1. For the absorption of non-specific haemagglutinins in Coombs reagent both A_1 and O cells were used. None of the cells used were over four weeks old. All cells were treated to six successive washings, in each case in a large volume of 0.85% saline containing 0.1% sodium azide. Azide was omitted in the preparations for immunizing rabbits.

Human Gamma Globulin

Human gamma globulin was kindly supplied by Dr. Bruce Chown, Director of the Rh Laboratory of the Winnipeg

General Hospital, and was found to be electrophoretically pure by Dr. W.P. McKenley of the Federal Department of Food and Drug, Ottawa.

Preparation of Coombs Reagents

The eluted antibody was prepared by the technique of Landsteiner and Miller (31). Sera from which antibody was eluted were that of Halvorson, Penner and Boadway. A measured volume of serum was inactivated in a 56°C. water-bath for half an hour and an equal volume of washed, packed A2 cells was added. The serum-cells mixture was incubated at 37°C. for two hours with shakings at 15 minute intervals and then the cells were recovered by centrifugation at 3000 r.p.m. for six minutes. A volume of saline, which was equal to the measured volume of serum, was added to the sensitized cells which had been previously washed four times with ice-cold saline. The saline-cells mixture was agitated gently in a 56°C. water-bath for five minutes; the heating served to elute antibodies from the surface of the cells to the saline. Finally, the eluted antibodies were recovered by centrifugation at 3000 r.p.m. for four minutes in a cup filled with water at 56°C. The method for immunization of rabbits was was that of Kabat (25). Antibody for immunization was freshly eluted each week and a portion from each elution was pooled. The pooled eluted antibodies were titrated with antiglobulin serum AHG "D" (6). Antisera prepared were anti-eluate-anti-A eluted from Halvorson serum (AHG "A" Halvorson), eluted from Penner serum (AHG "A" Penner), eluted from Boadway cord serum (AHG "A" Boadway) and anti-human-gamma-globulin (AHG) (6).

Treatment of Antiglobulin Sera before use

The Coombs reagents were heated in a 1:10 dilution of saline at 70°C. in a water-bath for 60 minutes with shaking at regular intervals to remove natural haemagglutinins. The process also served to inactivate the complement.

In most cases, the Coombs reagents were found to contain non-specific saline agglutinins which required the absorption of group O and group A_1 red cells. The Coombs sera were first diluted 1:10, heated at 56°C. for 30 minutes, and then absorbed with an equal volume of washed, packed red cells. The serum-cells mixture was allowed to stand at 4°C. for an hour with frequent shakings and then centrifuged at 2200 r.p.m. for six minutes. The procedure was repeated until complete absorption was attained.

Antiglobulin Test

The technique recommended by the National Institute of Health (35) was adopted with a slight modification.

To serial dilutions of eluted antibody ranging from 1:4 to 1:256, equal volumes of 2% A_1 cells were added. A saline control was included. The mixture was incubated at 37°C. for two hours with shaking at fifteen minutes intervals. A cross titration was carried out by adding 0.1 ml. serial dilution of absorbed Coombs sera to 0.1 ml. of washed, sensitized cells from each dilution of eluate, and a saline control was included. Tubes were read after having been centrifuged at 1500 r.p.m. for two minutes.

"Neutralisation" with Human Gamma Globulin

Serial dilutions of sterile human gamma globulin was made ranging from 100 γ per 0.2 ml. to 1.56 γ per 0.2 ml. and a saline control was included. Serial dilutions of AHG "A" and AHG "B" ranging from 1:10 to 1:2560 were made. To each dilution of Coombs sera was added an equal volume of each dilution of gamma globulin solution. The mixture was incubated at 4°C. overnight before being used in the antiglobulin test.

Addition of Anti-human gamma globulin

To each tube of the antisera-gamma-globulin after overnight incubation 0.1 ml. of 1:10 dilution of AHG was added. A negative control with AHG and saline was included.

Quantitative Micro-estimation of Antibody to Gamma Globulin in Coombs Reagents

Cleaning of Glassware

All glassware including pipettes were first cleaned in soap solution and, after rinsing, in cleaning solution for 24 hours. They were thoroughly rinsed in water and finally in distilled water.

Preparation of Gamma Globulin Solution

The gamma globulin was dissolved in saline at a concentration of one mg. per ml. Methiolate was added to a final concentration of 0.01%. The solution was sterilized by Candle filtration and stored in a brown glass bottle at 10°C.

Treatment of Coombs reagents

Coombs sera were treated to remove natural haemagglutinins by absorbing with erythrocytes as previously described. Lipid was removed by centrifugation at 10,000 r.p.m. at 4°C. for two hours. The complement was removed by the method of Heidelberger (23), using 6.3 mg. egg albumin and 2.5 mg. anti-egg albumin N for each five ml. of Coombs sera.

Qualitative Precipitin

The procedure described by Martin (25) was adopted. To a constant volume of 1:10 diluted Coombs serum, an equal volume of each serial dilution of stock gamma globulin was added, the dilutions ranging from 1:1 to 1:7680. Two controls were included: Coombs serum without gamma globulin and non-immune rabbit serum with gamma globulin solution. The system was allowed to react at room temperature for one hour after which it was placed at 10°C. The test was allowed to proceed for 72 hours, and readings were made at 24 hour intervals. At each reading, tubes were centrifuged at 1500 r.p.m. for 10 minutes. Formation of macroscopic visible precipitates was considered a positive reaction.

A fine test was performed, with a smaller range of dilution, after the highest serial dilution of gamma globulin solution yielding a precipitate with a constant volume of Coombs serum was obtained. The dilution of gamma globulin for the fine test was selected to give the endpoint at the middle of a ten harmonic dilutions series. The fine test

gave the precise quantity of gamma globulin with which 0.5 of 1:10 diluted Coombs serum reacted.

The least quantity of gamma globulin which still yielded a precipitate was then used with progressively decreasing amounts of Coombs serum. By using the same procedure as used in the titration of gamma globulin, the greatest dilution of Coombs serum with the least amount of gamma globulin for the formation of visible precipitates was obtained. The amount of gamma globulin and the dilution of Coombs serum at this point was considered to represent the equivalent zone proportions.

Quantitative Precipitin

Nitrogen analysis of the precipitin on the prepared Coombs sera was carried out by the method of Heidelberger (22) with a slight modification. Five millilitres of undiluted Coombs serum was delivered into each of a series of eight tubes each containing a progressively increasing amount of gamma globulin. The equivalent zone proportions were reached in tube number four and duplicated in tube number five. The tubes were plugged with corks and were permitted to react at room temperature for one hour after which they were transferred to 10°C. where they were kept for seven days. The contents of the tubes were mixed twice daily by gentle rotations.

At the end of the seventh day, precipitation was considered complete and tubes were centrifuged at 2000 r.p.m.

at approximately 4°C. for one hour. The supernatant from each tube was carefully decanted by slowly inverting the tube with a continuous motion. During decanting, the supernatant was observed closely to detect dislodged particles, in which case, the supernatant was recentrifuged to recover the lost precipitate. The packed precipitates were broken up by sharply tapping the tubes with fingers. Two to four drops of saline were added and mixed with the precipitates by repeated tapping. The precipitates were transferred to micro-Kjeldahl flasks. A small stirring rod was introduced into the tubes and the transfer was completed by washing the entire inner surface with several drops of N/1 NaOH followed by a few small portions of water.

Ten ml. micro-Kjeldahl flasks were used for digestion. After introducing samples, a few crystals of K_2SO_4 and 1.0 ml of concentrated H_2SO_4 saturated with $CuSO_4$ were added. The substrate was subjected to steam distillation to liberate NH_3 which was collected in 10 ml. of a saturated boric acid mixed indicator sulution. The amount of NH_3 was determined by titration with N/100 HCl.

Graphing of Precipitin N

The milligram nitrogen (mg N) of precipitate was obtained by multiplying the volume of N/100 HCl by 0.014 (a constant). The mg N of Gamma Globulin added was plotted against the total mg N of precipitate per ml of Coombs reagents. The mean of each pair of duplicating Coombs sera

was taken as the theoretical value. To test the fit between the mean curve so obtained and the curve produced on each animal, a Chi Square test was performed. If the fit was found to be satisfactory, the curve of the means was considered to represent the observed values of duplicate Coombs reagents.

R E S U L T S

RESULTS

Reaction of Eluted anti-A with the Coombs Reagent

Anti-A eluted from Halvorson serum, coated on A_1 cells, gave no reaction with Coombs reagent to human gamma globulin AHG, but agglutinations were observed, in each case, with the following Coombs reagents: Coombs serum to natural anti-A AHG "A" (Halvorson), Coombs serum to immune anti-A AHG "A" (Penner), Coombs serum to anti-A eluted from placenta cord serum AHG "A" (Broadway), and Coombs serum to anti-D AHG "D" (Briston). Titres of these Coombs reactions, done in cross-titrations, are shown in Table 1A. With the exception of AHG "A" (Halvorson) series and AHG "D" series, a greater amount of Coombs antibody was required to produce agglutination when a smaller amount of anti-A was available for sensitization. However, in the AHG "A" (Halvorson) system, the inversely proportional relationship between the quantity of Coombs antibody and that of the sensitizing human antibody was not evident as a 1/40 dilution of Coombs serum agglutinated all dilutions of eluate between the range of 1/16 to 1/256. It is difficult to interpret this discrepancy except as possible presence of non-specific agglutinins since upon repetition, different titres were obtained. In the AHG "D" system, two endpoints were observed; a 1/4 dilution of eluate gave a positive reaction with Coombs serum diluted 1/160, and a 1/128 dilution of eluate gave a positive reaction with Coombs serum diluted 1/640 suggesting the possible presence of two antibody systems. As in the case of Halvorson

eluate, anti-A eluted from three donors, Wong, Turbovsky and Baydack showed no reaction with AHG.

Immune anti-A eluted from Penner serum coated on A_1 cells gave positive indirect antiglobulin tests with the five prepared Coombs reagents including AHG. Titres of these Coombs reactions are given in Table 1B.

Anti-A eluted from Broadway placenta cord serum coated on A_1 cells gave positive indirect antiglobulin tests with the three Coombs reagents tested: AHG, AHG "A"(Halvorson), AHG "A" (Penner) as shown in Table 1C. The cord serum eluate gave similar titres with Coombs reagent AHG and Coombs reagent AHG "A" (Halvorson), but showed a four fold increase in titre with AHG "A" (Penner). However, no reaction with AHG was observed when cord serum absorbed with O cells was substituted although the absorbed cord serum gave a positive and increased titre to AHG "A" (Halvorson) (Table 1D).

The addition of Human Gamma Globulin on AHG "A" (Halvorson)

The addition of human gamma globulin to Coombs serum AHG "A" (Halvorson) showed an inhibitory effect on the ability of the Coombs serum to bring about agglutinations to A_1 cells sensitized by eluted anti-A. The neutralizing effect was observed with all the eluates tested: eluate from Halvorson serum (Table 2A), from Wong serum (Table 2B), from Turbovsky serum (Table 2C) and from Baydack serum (Table 2D). A similar neutralizing effect was shown by human gamma globulin on AHG "A" (Halvorson) when tested with A_1 cells sensitized with absorbed Broadway cord serum instead of eluate (Table 2E).

The Addition of AHG on the AHG "A" (Halverson) treated with Gamma Globulin

The results showed that the addition of AHG activated the AHG "A" (Halverson) which had been neutralized with gamma globulin. No effect on the agglutinating ability was observed by the addition of AHG to AHG which had been similarly treated with human gamma globulin (Table 3F). Titres of the reactivated Coombs serum given to A_1 cells sensitized by eluate from different natural anti-A sera are shown in Table 3A - 3E.

Precipitin Contents of the Coombs Sera

The precipitin contents of the Coombs sera were measured by the highest dilutions of the Coombs sera which yielded precipitates with the least amount of gamma globulin. The reciprocals of the dilution are expressed as titres of the Coombs sera showing in Table 4.

Quantitative Precipitin

The milligram nitrogen of the total precipitin per ml. of Coombs serum precipitated by varying amounts of human gamma globulin are shown in Table 5. At maximum precipitation endpoints, the ratio of the antibody nitrogen/gamma globulin nitrogen of the Coombs sera are found to be as follows: in the AHG series, the ratio of rabbit serum No. 8 was 1.55 and No. 9 was 1.37; in the AHG "A" (Halverson) series No. 33 was 4.07 and No. 34 was 2.97; in the AHG "A" (Penner) series No. 31 was 0.85 and No. 32 was 1.08; and in

the AHG "A" (Boadway), where only one animal No. 37 was immunized, the ratio was 8.92.

The values from Table 5 are shown in graphic form in Figure 1 to Figure 5. With the exception of AHG "A" (Boadway) pairs of duplicating Coombs sera are shown with their respective mean curves from Figure 1 to Figure 3. Since the probability of fit was found to be more than 90%, in each case, when the Chi Square tests were performed on the curves of each pair of duplicating Coombs sera with their respective mean curve, the mean values were considered valid representation. The mean ratio of antibody nitrogen/gamma globulin nitrogen at maximum precipitation endpoints are found to be as follows; 1.46 for AHG; 3.52 for AHG "A" (Halvorson); 0.965 for AHG "A" (Penner); and 8.92 for AHG "A" (Boadway).

TABLES

TABLE 1

CROSS TITRATION OF ELUTED ANTI-A COATED ON A_1 CELLS WITH ANTIGLOBULIN

1A ELUTED ANTI-A HALVORSON

Antiglobulin Sera	Dilution of eluted anti-A (Halvorson) for sensitization							
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	saline
AHG	0_i	0	0	0	0	0	0	0
AHG "D" (Briston)	160_{ii}	40	80	160	640	640	80	0
AHG "A" (Halvorson)	320	160	40	40	40	20	40	0
AHG "A" (Fennor)	640	320	320	40	0	0	0	0
AHG "A" (Boadway)	160	160	20	0	0	0	0	0

i no observable macroscopic agglutination

ii reciprocal of highest dulution of antiserum showing agglutination of sensitized red cells expressed as titre.

1B ELUTED ANTI-A PENNER

Antiglobulin Sera	Dilution of eluted anti-A (Penner) for sensitization							
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	saline
AHG	2560	2560	2560	80	0	0	0	0
AHG "D" (Briston)	2560	2560	2560	40	40	40	0	0
AHG "A" (Halvorson	1280	640	40	40	0	0	0	0
AHG "A" (Penner)	2560	320	320	20	20	0	0	0
AHG "A" (Boadway)	160	40	0	0	0	0	0	0

1C ELUTED ANTI-A BOADWAY

	Dilution of eluted anti-A (Boadway) for sensitization							
Antiglobulin Sera	1:4	1:8	1:16	1:32	1:64	1:128	1:256	saline
AHG	80	40	40	0	0	0	0	0
AHG "A" (Halvorson)	40	40	40	0	0	0	0	0
AHG "A" (Penner)	80	80	40	80	20	0	0	0

1D ANTI-A ABSORBED BOADWAY

	Dilution of absorbed Boadway cord anti-A for sensitization							
Antiglobulin Sera	1:4	1:8	1:16	1:32	1:64	1:128	1:256	saline
AHG	0	0	0	0	0	0	0	0
AHG "A" (Halvorson)	128	128	128	32	16	16	0	0

TABLE 2

REACTION OF ELUTED ANTI-A, DILUTED 1:20 COATED ON A_1 CELLS WITH COOMBS AND "A" TREATED WITH HUMAN GAMMA GLOBULIN

2A ELUTED ANTI-A HALVORSON

	Dilution of ANC "A" (Halvorson)									
γ Globulin µg/0.2 ml.	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	saline
100	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
12.5	+	+	-	-	-	-	-	-	-	-
6.25	+	+	+	-	-	-	-	-	-	-
3.12	+	+	-	-	-	-	-	-	-	-
1.56	+	+	+	-	-	-	-	-	-	-
Control 0	+	+	+	+	+	+	-	-	-	-

2B ELUATED ANTI-A WONG

	Dilution of AHG "A" (Halverson)									
γ Globulin mg/0.2 ml.	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	saline
100	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
Control 0	+	+	+	+	+	-	-	-	-	-

20 MUTED ANTI-A TURBOVSKY

γ Globulin µg/0.2 ml.	Dilution of AHG "A" (Halvorson)									
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	Saline
100	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
12.5	+	+	-	-	-	-	-	-	-	-
Control 0	+	+	+	+	+	+	-	-	-	-

2D ELUTED ANTI-A RAYBACK

	Dilution of ANG "A" (Halvorson)									
γGlobulin µg/0.2 ml	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	saline
100	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
Control 0	+	+	+	+	-	-	-	-	-	-

2% ABSORBED BOARDAT CORD SERUM

	Dilution of ABS "A" (Halvorson)							
γ Globulin mg/0.2 ml.	1:4	1:8	1:16	1:32	1:64	1:128	1:256	saline
100	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-
3.12	+	+	+	-	-	-	-	-
1.56	+	+	+	-	-	-	-	-
Control 0	+	+	+	+	-	-	-	-

TABLE 3

REACTION OF ELUTED ANTI-A_1, DILUTED 1:20, COATED ON A_1 CELLS WITH COOMBS ANG "A" TREATED WITH GAMMA GLOBULIN ADDED WITH ANG 1:10 DILUTED 3A ELUTED ANTI-A HALVORSON

	Dilution of AHG "A" (Halvorson)										
γ Globulin mg/0.2 ml.	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	CC	saline
100	+	+	+	+	+	+	-	-	-	-	-
50	+	+	+	+	+	+	-	-	-	-	-
25	+	+	+	+	+	-	-	-	-	-	-
12.5	+	+	+	+	-	-	-	-	-	-	-
6.25	+	+	+	+	+	+	+	-	-	-	-
3.12	+	+	+	+	+	+	+	-	-	-	-
1.56	+	+	+	+	+	+	-	-	-	-	-
Controls AHG "A"	+	+	+	+	+	+	-	-	-	-	-

3B ELUTED ANTI-A NONG

γ Globulin µg/0.2 ml.	Dilution of AHG "A" (Halvorsen)										
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	GG	saline
100	+	+	+	+	+	+	-	-	-	-	-
50	+	+	+	+	+	-	-	-	-	-	-
25	+	+	+	+	+	+	-	-	-	-	-
12.5	+	+	+	+	+	-	-	-	-	-	-
Controls AHG "A"	+	+	+	+	+	+	-	-	-	-	-
AHG "A" and AHG	+	+	+	+	+	-	-	-	-	-	-

3C ELUTED ANTI-A TURBOVSKY

γ Globulin µg/0.2 ml.	Dilution of AHG "A" (Halvorson)										
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	GG	saline
100	+	+	+	+	+	+	+	-	-	-	-
50	+	+	+	+	+	-	-	-	-	-	-
25	+	+	+	+	+	+	+	-	-	-	-
12.5	+	+	+	+	+	+	-	-	-	-	-
Controls AHG "A"	+	+	+	+	+	+	-	-	-	-	-
AHG "A" and AHG	+	+	+	+	+	+	+	-	-	-	-

3D ELUTED ANTI-A BAYDACK

γ Globulin µg/0.2 ml.	Dilution of AHG "A" (Halvorson)										
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	GG	saline
100	+	+	+	+	+	+	+	+	-	-	-
50	+	+	+	+	+	+	+	+	-	-	-
25	+	+	+	+	+	+	+	-	-	-	-
12.5	+	+	+	+	+	+	+	+	-	-	-
Controls AHG "A"	+	+	+	+	+	-	-	-	-	-	-
AHG "A" and AHG	+	+	+	+	+	-	-	-	-	-	-

3E ABSORBED CORD SERUM

γ Globulin µg/0.2 ml.	Dilution of AHG "A" (Halvorson)								
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	CC	saline
100	+	+	+	-	-	-	-	-	-
50	+	+	+	+	-	-	-	-	-
25	+	+	+	+	-	-	-	-	-
12.5	+	+	+	-	-	-	-	-	-
6.25	+	+	+	+	-	-	-	-	-
3.12	+	+	+	-	-	-	-	-	-
1.56	+	+	+	-	-	-	-	-	+
Controls AHG "A"	+	+	+	-	-	-	-	-	-
AHG "A" AND AHG	+	+	+	+	-	-	-	-	-

TABLE 3F

A_1 Cells sensitized with 1:20 dilution of eluate of Halvorson serum and tested by the Indirect Coombs Technique with AHG that had been mixed with Gamma Globulin allowed to stand at 4°C. overnight and 0.1 ml. of 1:10 diluted AHG was added just before testing.

	Dilution of AHG										
µg/0.2 ml. of γ Globulin	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	CC	saline
100	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-	-
Controls AHG	-	-	-	-	-	-	-	-	-	-	-
AHG "A"	+	+	+	+	+	-	-	-	-	-	-

TABLE 4

QUALITATIVE PRECIPITIN TEST ON COOMBS SERA

Rabbit No.	Coombs Antiserum to	Titre of Antisera in Equivalent zone	Antigen added mg γ Globulin
8	γ Globulin	42_1	.000976
9	γ Globulin	24	.000976
31	Penner eluate immune anti-A	44	.002728
32	Penner eluate immune anti-A	35	.003428
33	Halvorson eluate Natural anti-A	25	.003200
34	Halvorson eluate Natural anti-A	20	.005000
37	Roadway cord eluate immune anti-A	32	.000029

1. Reciprocal of the highest dilution of antisera which gave precipitates with the least amount of antigen expressed as titre.

TABLE 5

MICRO-ESTIMATION OF THE NITROGEN CONTENT OF PRECIPITIN

5A PRECIPITIN TO HUMAN GAMMA GLOBULIN

Antiserum No.	mg N of γ Globulin added per ml of Coombs serum	mg N of the total precipitin per ml of Coombs serum
8	1.226	0.220
	1.020	2.198
	0.919 1	2.450
	0.919 1	2.492
	0.817	1.792
	0.715	1.498
	0.613	1.428
9	1.328	0.560
	1.226	1.820
	1.022 1	2.400
	1.022 1	2.450
	0.816	1.540
	0.715	1.456
	0.511	1.400
mean value of No.8 & No.9	1.328	0.280
	1.226	1.023
	1.020	2.324
	0.919 1	2.486
	0.916	1.666
	0.715	1.477
	0.613	1.428
	0.511	1.400

1. Equivalent zone

5B PRECIPITIN TO IMMUNE ANTI-A

Antiserum No.	mg N of γ Globulin added per ml of Coombs serum	mg N of the total precipitate per ml of Coombs serum
31	1.120	0.690
	0.809	0.850
	0.784	0.900
	0.728	1.080
	0.672$_i$	1.240
	0.672$_i$	1.260
	0.616	1.400
	0.448	0.840
32	1.0250	0.700
	0.8176	0.868
	0.7154	1.092
	0.6132$_i$	1.260
	0.6132$_i$	1.302
	0.5110	0.980
	0.4088	0.840
	0.2044	0.714
mean value of No.31 & No.32	1.025	0.700
	0.817	0.856
	0.809	0.876
	0.784	0.925
	0.728	1.034
	0.715	1.036
	0.672	1.220
	0.613$_i$	1.236
	0.511	0.975
	0.448	0.860
	0.408	0.820

i. Equivalent zone

SC PRECIPITIN TO NATURAL ANTI-A

Antiserum No.	mg N of γ Globulin added per ml of Coombs serum	mg N of the total precipitate per ml of Coombs serum
33	0.817	0.840
	0.613	1.120
	0.511	1.260
	0.408[1]	2.040
	0.408[1]	2.100
	0.316	1.680
	0.204	1.400
	0.102	0.402
	0.081	0.280
34	1.022	0.910
	0.817	0.980
	0.613	1.330
	0.560[1]	1.600
	0.560[1]	1.600
	0.408	1.260
	0.214	0.980
	0.102	0.700
	0.081	0.200
mean value of No. 33 & No. 34	0.817	0.910
	0.613	1.225
	0.560	1.385
	0.511	1.420
	0.408[1]	1.680
	0.316	1.390
	0.214	1.205
	0.204	1.180
	0.102	0.501
	0.081	0.250

1. Equivalent zone

UNIVERSITY OF MANITOBA LIBRARY

5D PRECIPITIN TO PLACENTA CORD ANTI-A

Antiserum No.	mg N of γ Globulin added per ml of Coombs serum	mg N of the total precipitin per ml of Coombs serum
37	0.102 0.071 0.061 0.051 i 0.051 i 0.041 0.020	0.280 0.700 0.896 1.036 1.008 0.420 0.280

i. Equivalent zone

FIGURES

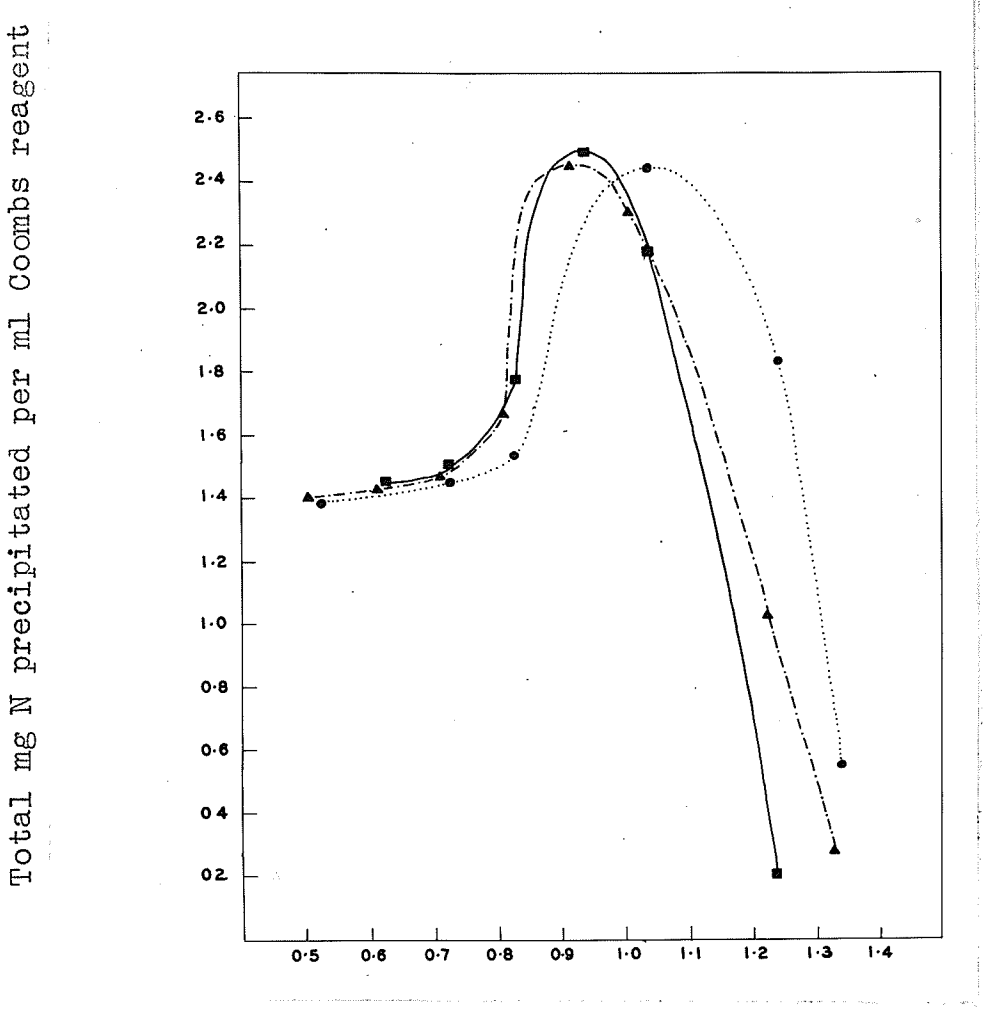


Figure 1. Micro-estimation of the nitrogen content of the precipitates formed by the Coombs sera AHG with varying amount of γ globulin. Antiserum No. 8, ● ; antiserum No. 9, ■ ; mean value of antisera ▲ .

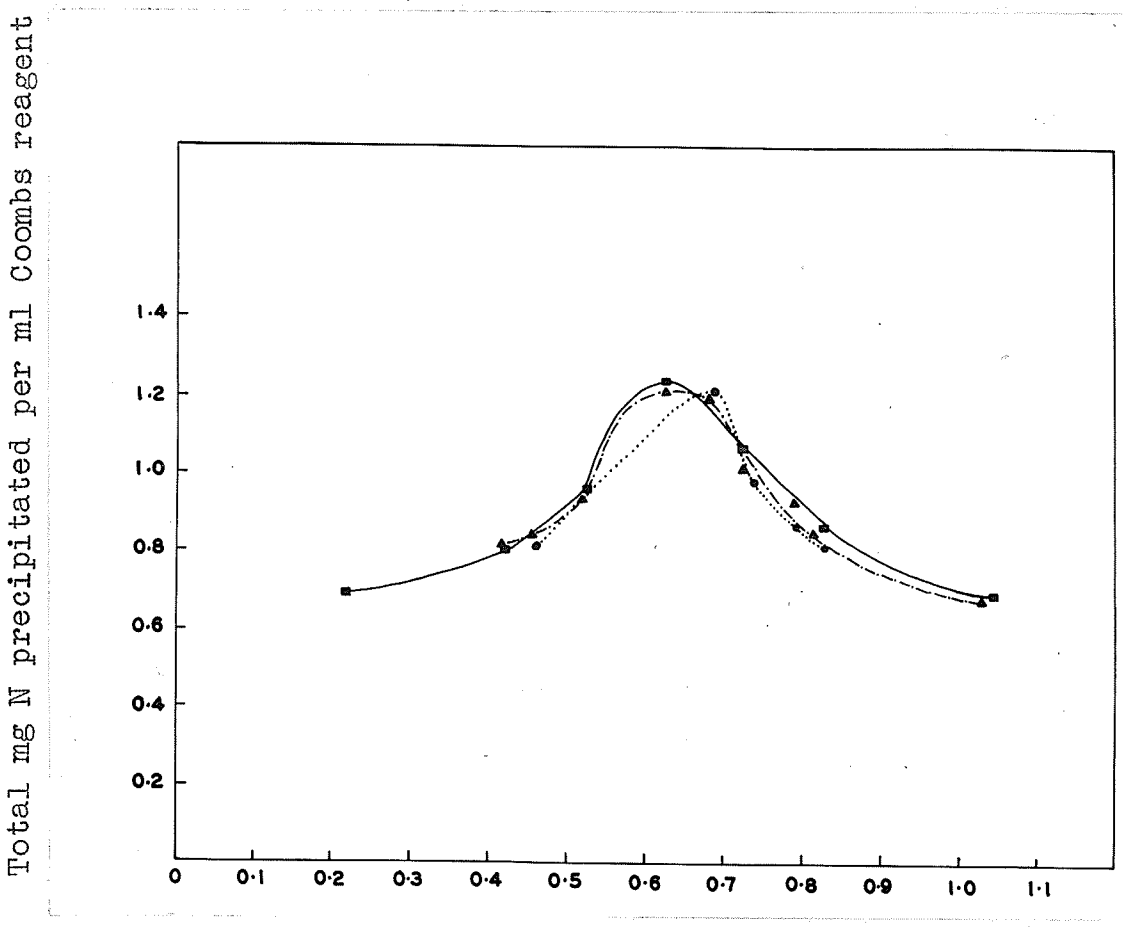


Figure 2. Micro-estimation of the nitrogen content of the precipitates formed by the Coombs sera AHG "A" (Penner) with varying amount of γ globulin. Antiserum No. 31, ● ; antiserum No. 32, ■ ; mean value of antisera ▲ .

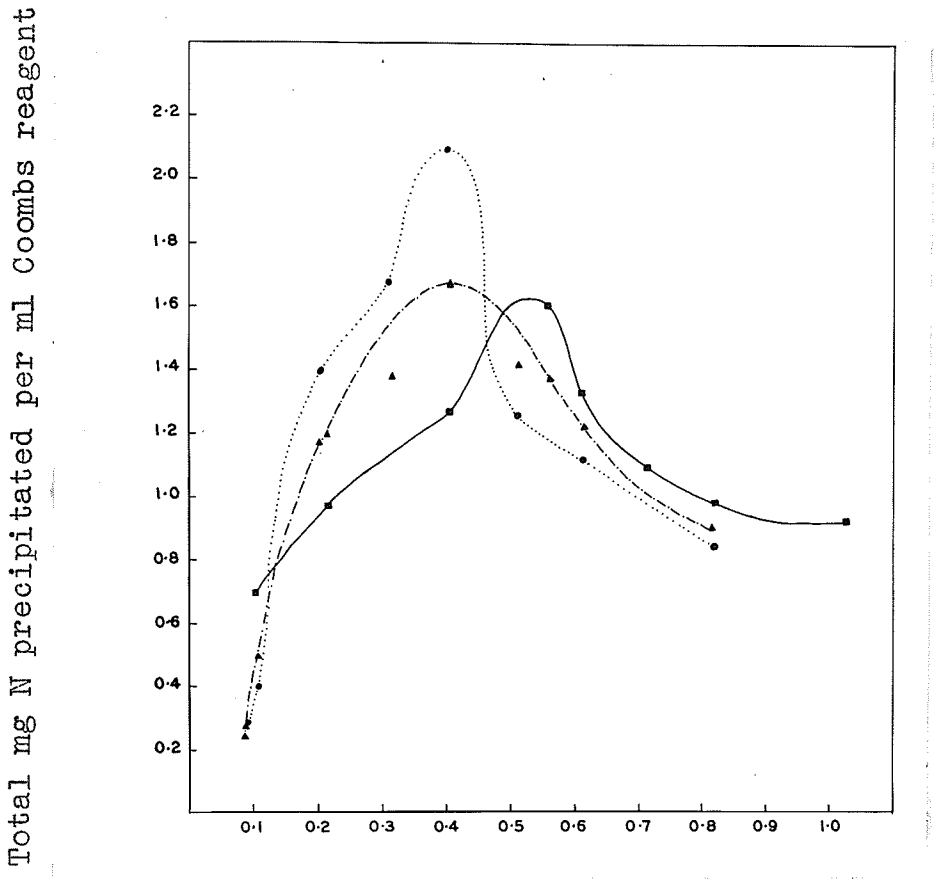


Figure 3. Micro-estimation of the nitrogen content of the precipitates formed by the Coombs sera AHG "A" (Halvorson) with varying amount of γ globulin. Antiserum No. 33, ● ; antiserum No. 34, ■ ; mean value of antisera▲ .

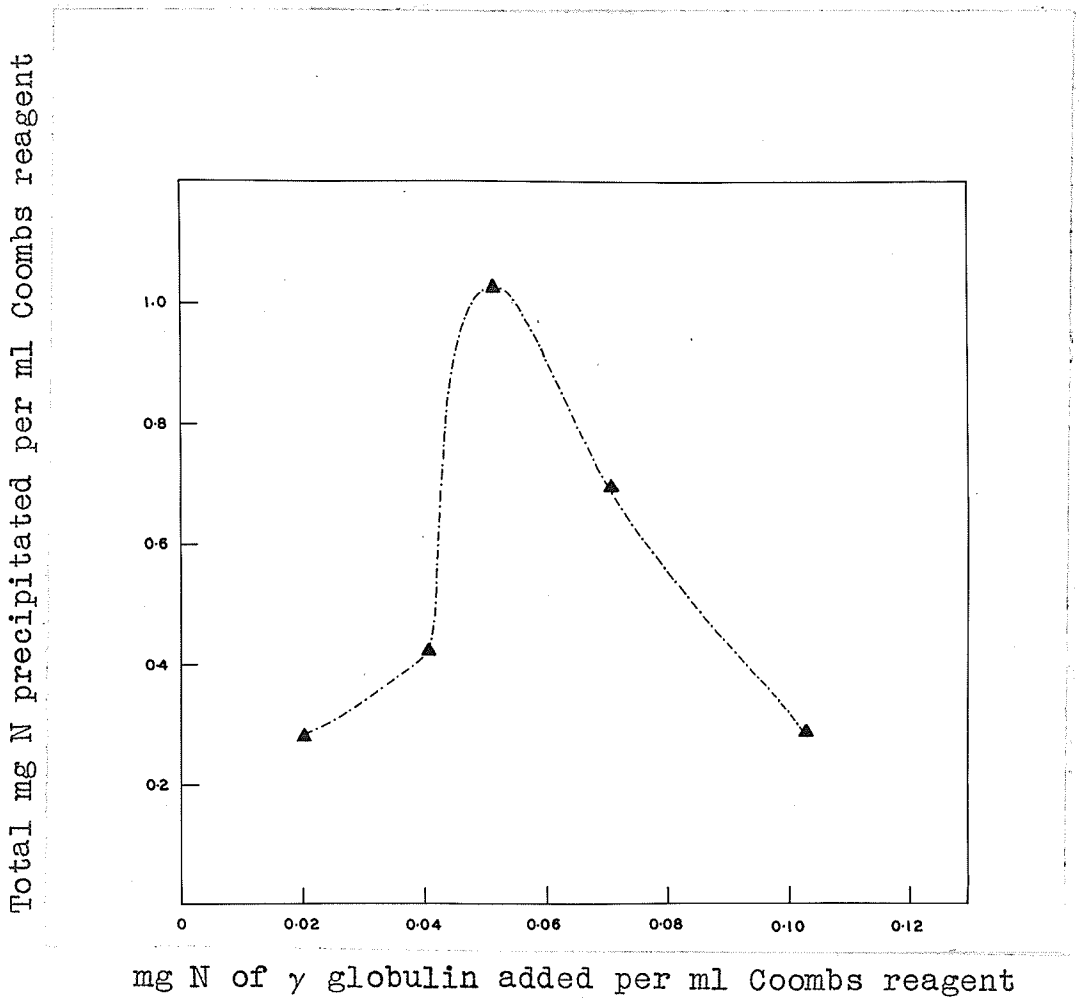


Figure 4. Micro-estimation of the nitrogen content of the precipitates formed by AHG "A" (Boadway) with varying amount of γ globulin.

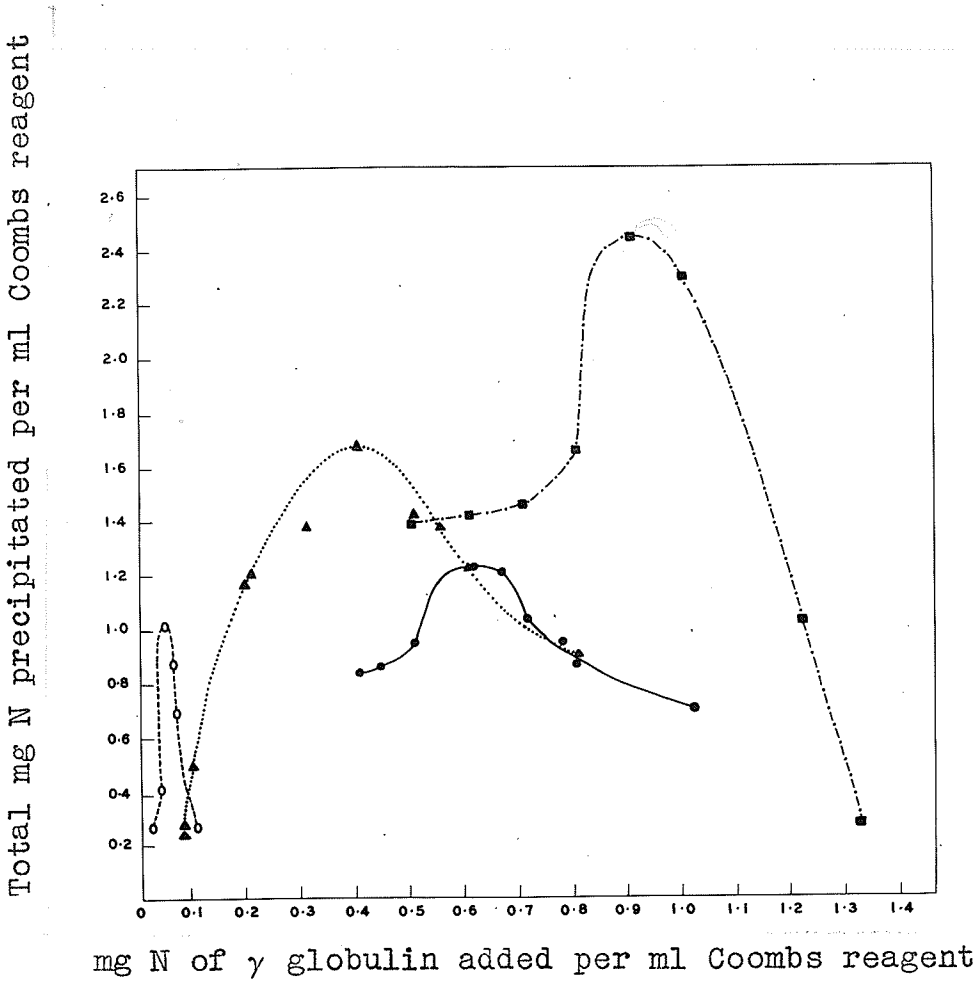


Figure 5. Micro-estimation of the nitrogen content of the precipitates formed by all the prepared Coombs sera with varying amount of γ globulin. AHG, ■ ; AHG "A" (Halvorson), ▲ ; AHG "A" (Penner), ● ; AHG "A" (Boadway) O .

DISCUSSION

DISCUSSION

Since gamma globulin exhibited an inhibitory effect on the agglutination of A_1 cells which were coated with eluted natural anti-A from Halvorson serum and then suspended in Coombs serum to the Halvorson natural anti-A, AHG "A", it may be inferred that the human antibody, or the Coombs antibody, or both, is susceptible to the action of gamma globulin neutralization resulting from the masking of the anti-gamma globulin reacting sites. Since it has been shown by McDuffie et al. (34) that human anti-A isoagglutinins are themselves composed in part of gamma globulin, it follows that the anti-gamma-globulin component must belong to the Coombs antibody. It is evident therefore that there may exist an anti-gamma globulin reacting site in AHG "A" (Halvorson).

While Weiner et al. (42) reported a direct proportional relationship between the agglutinating ability of an antiserum and its precipitin nitrogen content to the corresponding specific antigen, the present work shows no such correlation between the agglutinating ability of a Coombs serum and its precipitin nitrogen content to gamma globulin. Coombs serum to gamma globulin (AHG) gave a negative antiglobulin test to the natural occurring anti-A. Yet, the precipitin nitrogen content to gamma globulin of AHG is higher than AHG "A" (Penner) and lower than AHG "A" (Halvorson) both of which gave a positive antiglobulin test to the same

natural occurring anti-A isoagglutinin. The fact that the agglutinating ability of gamma globulin treated AHG "A" (Halvorson) was restored by the addition of AHG indicates the presence of an active group in the neutralized serum which reacts with the non-gamma globulin components of the sensitizing human isoagglutinin, and which will be referred to as an anti-non-gamma-globulin component. It appears that the natural occurring anti-A isoagglutinin possesses both gamma and non-gamma globulin determinant groups both of which must be "accepted" before agglutinations occur. Since the AHG alone failed to bring about agglutination of A_1 cells sensitized with natural occurring anti-A, it is evident that the AHG does not possess an anti-non-gamma globulin component.

Since AHG gave positive antiglobulin tests to both immune anti-A from the Penner serum and from the Broadway cord serum, the active determinant group of the immune isoagglutinins appears to be confined to the gamma globulin component. However, the fact that antisera to the immune anti-A gave positive antiglobulin tests to natural occurring anti-A also indicates the presence of an anti-non-gamma globulin component in these Coombs sera. These observations suggest the presence of two forms of isoagglutinins in the immune sera: one having only a gamma globulin determinant group responsible for the positive antiglobulin test with AHG; and one having both gamma and non-gamma globulin determinant groups which stimulate the formation of antibody possessing the corresponding reacting sites. The present

findings are not inconsistent with that of Cutbush (11) who reported that immune anti-A was mainly but not wholly gamma globulin. The assumption that all isoagglutinins of the natural occurring anti-A possess both gamma and non-gamma determinant groups while only part of the isoagglutinins of the immune form of anti-A possesses the two would necessarily mean a greater proportion of the non-gamma globulin components in the natural occurring anti-A sera. In this respect, the present work is supported by the findings of Abelson (1). It is interesting to note Kabat's observation that the proportion of components of lower sedimentation constant (non-gamma globulin components) in horse anti-pneumococcal antibodies increases with prolonged immunization (24).

Since the amount of gamma globulin, in terms of milligram nitrogen, rendering one milligram Coombs serum AHG "A" (Halvorson) inactive to eluted natural anti-A was found to be within the range of 0.319 to 0.639, with a mean value of 0.474, while the amount of gamma globulin for the maximal precipitation of one milligram of AHG "A" (Halvorson) was within the range of 0.408 to 0.560 with a mean value of 0.484, it is evident that all the anti-gamma globulin components in the Coombs antibody are required for agglutination. On the other hand, to render the same Coombs serum inactive to anti-A isoagglutinins from cord serum, the amount of gamma globulin required was within the range of 0.319 to 0.015, the mean value of which was 0.167. This indicates approximately one third of the anti-gamma globulin component in the Coombs antibody take part in the agglutination

of cells sensitized by anti-A isoagglutinins from the cord serum. Since the cord isoagglutinins react with AHG in contrast to the Halvorson isoagglutinins which fail to react with AHG, it would appear likely that only one third of Halvorson Coombs antibody are anti-gamma globulin similar to those in AHG while the remaining two thirds are anti-gamma globulins which react only with the natural anti-A iso-agglutinins. A comparison of the anti-gamma globulin contents of the Coombs sera substantiates this observation. In terms of milligram nitrogen the value of anti-gamma globulin content for AHG was 1.46, approximately one third of that for AHG "A" (Halvorson), the value of which was 3.52. Since only one third of the Coombs antibody in AHG "A" (Halvorson) is specific for the isoagglutinins in the cord serum, a low dilution of Coombs reagent would be required for a positive antiglobulin test. The present results show that for a positive antiglobulin test, a 1/16 diluted AHG "A" (Halvorson) was required for the isoagglutinins from the cord serum comparing to a 1/320 dilution for the isoagglutinins of the natural occurring anti-A.

Because of the limited amount of Boadway cord serum available, antiglobulin tests were not performed on these immune isoagglutinins with neutralized Coombs sera and no interpretation could be made on the high precipitin content to gamma globulin of the antiserum to the cord isoagglutinins.

CONCLUSION

CONCLUSION

The present investigation shows that natural anti-A isoagglutinins may be distinguished from the immune form by means of antiglobulin test with Coombs serum to purified gamma globulin. There is evidence that natural occurring anti-A is mainly, but not wholly, gamma globulin which is a functional component in a positive Coombs reaction.

Coombs sera prepared with natural occurring anti-A shows a similar reactivity as those prepared with immune anti-A isoagglutinins. However, only one-third of the anti-gamma globulin components in the Coombs antibodies prepared with natural occurring anti-A appears to be active with immune anti-A isoagglutinins.

R E F E R E N C E S

REFERENCES

(1) Abelson, N.M., and Rawson, A.J. (1959). Studies of Blood Group Antibodies. J. Immunol. 82: 435-443

(2) Boorman, K.E., Dodd, B.E., and Trinick, R.H. (1949). Haemolytic Disease of the Newborn due to anti-A antibodies. Lancet, 1: 1088-1091.

(3) Boorman, K.E., Dodd, B.E., and Mollison, P.L. (1945). Iso-immunization to Blood Group factor A, B and Rh. J. Path. Bact. 57: 157-169.

(4) Boorman, K.E., Dodd, B.E., and Morgan, W.T.J. (1945). Enhancement of action of immune haemagglutinins by human serum. Nature. 156: 663-664.

(5) Cater, D.B., and Loughrey, J.A. (1945). Method of demonstrating Rh agglutinins in cases of erythroblastosis fetalis. Amer. J. clin. Path. 15: 575.

(6) Chan, P.C. (1957). Antisera to gamma globulin and to eluted Rh antibody for the detection of incomplete Rh agglutinin. Thesis. University of Manitoba.

(7) Coombs, R.R.A., Mourant, A.E., and Race, R.R. (1945). Detection of weak and "incomplete" Rh agglutinins, a new test. Lancet. 2: 15-16.

(8) Crawford, H., Cutbush, Marie, and Mollison, P.L. (1953). Haemolytic disease of the newborn due to anti-A. Blood 8: 260.

(9) Crawley, L.V., and Bowroncle, B.A. (1951). Specificity of auto antibodies in acquired haemolytic anemia. Blood. 11: 700.

(10) Crunbach, A., and Gasser, C. (1948). ABO - Inkompatibilitaten und morbus Haemolyticus. Helv. paediat. acta. 3: 447. cited by Crawford, Cutbush, and Mollison. (1953). Blood. 8: 639.

(11) Cutbush, Marie, Crawford, H., and Mollison, P.L. (1955). Observation on anti-human globulin Sera. Brit. J. Haematology. 1: 410-421.

(12) Dienst, A. (1905). "Das Eklampsiegift". Zbl. Gynä k. 29: 353. cited by Prest, E., Bonnie, J.A., Simmons, R.T., and Newland, B.T.J. (1955). Haemolytic disease of the newborn due to ABO isosensitization in association with potent anti-A agglutinins. Med. J. Aust. 2: 153.

(13) Dodd, B.E. (1952). Linked anti-A and anti-B antibodies from Group O sera. Brit. J. exp. Path. 33: 1-18.

(14) Diamond, L.K., and Abelson, N.M. (1945). The demonstration of anti-Rh agglutinins an accurate and rapid slide test. J. Lab. clin. Med. 30: 204.

(15a) Erwin, D.M., Christian, R.M., and Young, L.E. (1950). Observation on Destruction of Recipient's A cells after transfusion of Group O blood containing High Titer of A antibodies of Immune type not easily neutralizable by soluble A substance. Blood. 5: 553

(15b) Erwin, D.M., and Young, L.E. (1950). Dangerous Universal Donors. II Further Observations on In Vivo and In Vitro behavior of isoantibodies of Immune Type present in Group O. Blood. 5: 61.

(16) Furuhata, T. (1927). Heredity of Blood Groups. Jap. med. Wschr. 7: 197-208.

(17) Gitlin, D., and Edelhoch, H. (1951). Study of reaction between human serum albumin and its homologous equine antibody through medium of light scattering. J. Immunol. 66: 67-77.

(18) Goldberg, R.J., and Campbell, D.H. (1951). Light-scattering properties of antigen-antibody reaction. J. Immunol. 66: 79-86.

(19) Heidelberger, M., and Kendall, F.E. (1929). Quantitative study of precipitin reaction. Type III pneumococcus polysaccharide and purified homologous antibody. J.exp. Med 50: 809-823.

(20a) Heidelberger, M., and Kendall, F.E. (1936). Quantitative studies on antibody purification; dissociation of precipitates formed by pneumococcus specific polysaccharides and homologous antibodies. J. exp. Med. 64: 161-172.

(20b) Horsfall, F.L., Jr., Goodner, K., and Macleod, C.M. (1936). Type specific antipneumococcus rabbit serum. Science. 84: 579-581.

(20c) Heidelberger, M., and Kabat, E.A. (1938). Quantitative studies on antibody purification; dissociation of antibody from pneumococcus specific precipitate and specifically agglutinated pneumococci. J. exp. Med. 67: 181-199.

(21) Heidelberger, M., Kendall, F.E., and Scotleo, C.M. (1935). Quantitative studies on precipitin reaction. Antibody production in rabbits injected with azo protein. J. exp. Med 58: 137-152. 61: 559.

(22) Heidelberger, M., and Macpherson, C.F.C. (1943). Quantitative micro-estimation of antibodies in sera of man and other animals. Science. 98: 63.

(23) Heidelberger, M., and Mayer, M. (1942). Quantitative chemical studies on complement on alexin; addition of human complement to specific precipitates. J. exp. Med. 75: 285-292.

(24) Kabat, E.A. (1943). as cited by Abelson, N.M., and Rawson, A.J. in Ref. (1). J. Immunol. 47: 513.

(25) Kabat, E.A. (1948). Experimental Immuno Chemistry. C.C. Thomas, Springfield, Ill. (pp. 543).

(26) Kabat, E.A. (1956). Blood Group Substances. Their chemistry and immunology. Academic Press Inc., N.Y. (pp. 46, 269).

(27) Landsteiner, K. (1900). Zur Kenntirs der Anti fermentativen bytischen und agglutinierenden wirkungen des blutserum und der lympho. Zbl. Bakt. 27: 357.

(28) Landsteiner, K., and Levine, P. (1927). A new agglutinable factor differentiating individual human bloods. Proc. Soc. expe. Biol. 24: 600.

(29) Landsteiner, K., and Levine, P. (1927). Further observations on individual differences of human blood. Proc. Soc. exp. Biol. 24: 941.

(30) Landsteiner, K., and Weiner, A.S. (1940). An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc. Soc. exp. Biol. 45: 223.

(31) Landsteiner, K., Miller, C.P. (1925). Serological studies on the blood of primates. II The blood groups in anthropoid apes. J. exp. Med. 42: 852.

(32) Mollison, P.L. (1954). Personal communication cited by Prest, E., Donnie, J.A., Simmins, R.T., and Newland, B.T.J. (1955). Haemolytic disease of the newborn due to ABO iso-sensitization in association with potent anti-A agglutinins. Med. J. Aust. 2: 153.

(33) Mollison, P.L., and Cutbush, Marie. (1949). Haemolytic Disease of the Newborn due to Anti-A antibodies. Lancet. 2: 173.

(34) McDuffie, F.C., Kabat, E.A., Allen, P.Z., and William, C.A.Jr. (1956). An immunochemical study of the relationship of human blood group isoantibodies. to γ-1 and γ-2 globulins. J. Immunol. 81: 48-64.

(35) National Institutes of Health. (1949). Minimum Requirements for anti-human serum for anti-globulin test. Federal Security Administration, Washington, D.C.

(36) Moreschi, C. (1907). Ueber hamolyse beschlennigende immunsubstanzen. Zbl. Bakt. 46: 346-352. as cited by Richard, E., Rosenfield, R.E., and Vogel, P. (1951) in Am. clin. Path. 21: 301-318.

(37) Race, R.R. (1944). "Incomplete" antibody in human serum. Nature. 153: 560.

(38) Race, R.R., and Sanger, R. (1950). Blood Groups in Man. Oxford, Blackwell Scientific Pub.

(39) Reepmaker, J., and van Loghem, J.J. (1953). vox sanguinis 3; 143. as cited by Kabat, E.A. (1956) in the Blood Group Substances. Academic Press Inc., Pub., New York. 261.

(40) Robinson, G.C., Philips, R.M., and Pryetowsky, M. (1951). Spherocytosis fetalis associated with ABO incompatibility. Pediat. 7: 164.

(41) Rosenfield, R.E. (1955). A-B hemolytic disease of the newborns analysis of 1480 cord blood specimens, with special reference to the direct anti-globulin test and to the group O mother. Blood. 10: 17.

(42) Weiner, A.S. (1944). A new test (blocking test) for Rh sensitization. Proc. Soc. exp. Biol. 56: 173.

(43) Weiner, A.S. (1941). Subdivision of Group A and Group AB. II. Isoimmunization of Ag with special reference to the role of the sub-groups in Transfusion Reactions. J. Immunol. 41: 181-199.

(44) Witebsky, E. (1948). Interrelationship between Rh system and AB system. Blood. Special issue. 2: 66-79.