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# Quantitative Immunology of Immune Hemolytic Anemia

## II. THE RELATIONSHIP OF CELL-BOUND ANTIBODY TO HEMOLYSIS AND THE EFFECT OF TREATMENT

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**ABSTRACT** The concentration of cell-bound and serum antibody was determined in a series of patients with warm antibody immune hemolytic anemia by determining the amount of C $\bar{1}$  fixed to the cells by anti-IgG. This was compared to the rate of hemolysis as determined by hemoglobin concentration and reticulocyte count, or the endogenous production of carbon monoxide. The rate of hemolysis was, in general, proportional to the concentration of cell-bound antibody. In splenectomized patients, the rate of hemolysis was very much less than in unsplenectomized patients for a given concentration of cell-bound antibody. When prednisone was given, three effects were noted: (a) at high doses of drug, the concentration of cell-bound antibody decreased rapidly and the concentration of serum antibody increased, suggesting that the affinity of antibody for antigen had been altered; (b) in patients achieving remission, the concentration of serum antibody fell to low levels but rose again if the dose of prednisone was insufficient; (c) in one patient, prednisone appeared to inhibit sequestration of highly sensitized cells.

### INTRODUCTION

The commonest form of autoimmune hemolytic anemia is characterized by the presence of antibody of the IgG immunoglobulin class on the red cell (1). These antibodies characteristically react with red cell antigens at body temperature, and they fix complement relatively inefficiently since two antibody molecules in juxtaposition are required to initiate a complement sequence (2).

The exact mechanism by which the red cells are destroyed in this syndrome is not completely understood. Recently it has been demonstrated that the presence of the IgG antibody on the surface of the red cell may cause it to adhere to monocytes and splenic macrophages (3, 4). In this reaction, a portion of the red cell mem-

brane may be removed, resulting in spherocytosis. Sequestration of the cells by elements of the reticuloendothelial system, especially the spleen, is probably responsible for the ultimate destruction of the cells. When complement is fixed, direct cytolysis may account for a small part of the cellular destruction.

The rate of hemolysis in patients with hemolytic anemia due to warm-reacting antibody may vary greatly, and a relationship between the degree of anemia or the rate of hemolysis and the amount of antibody on the red cell surface has been suspected but has not been established (5, 6). The investigation of this relationship has been hampered by the difficulty in determining the amount of antibody on the cells and in the serum of patients with this disease. The amount of antibody on the cell has been estimated by elution (7), with radiolabeled anti-globulin (6) and, more recently, by an indirect estimate of the amount of anti-IgG which adheres to cells coated with IgG (8).

In the present studies, we have undertaken to estimate the amount of antibody present on the red cell surface by measuring the fixation of the first component of complement, C $\bar{1}$ . Since IgG autoimmune antibodies fix complement only very poorly, if at all, we have reacted IgG-coated cells with rabbit anti-IgG. The combination of two anti-globulin molecules brings about the fixation of a C $\bar{1}$  molecule (9). The concentration of anti-globulin can be adjusted so that the direct proportion exists between the number of C $\bar{1}$  molecules fixed and the number of IgG autoimmune antibody molecules present on the red cell. By this method, we are able to derive a minimum but proportional estimate of the number of antibody molecules present on the surface of the red cell.

The rate of hemolysis in these patients was measured by a modification<sup>1</sup> of the endogenous carbon monoxide

<sup>1</sup>Logue, G. L., W. F. Rosse, W. T. Smith, H. A. Salzman, and L. A. Gutterman. 1970. Endogenous carbon monoxide production measured by gas phase analysis: an estimation of heme catabolic rate. Submitted for publication.

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method of Coburn, Williams, and Kahn (10). This method was chosen since frequent serial estimates of the red cell survival time were needed in order to follow the effect of therapy. Using these methods, we have investigated the effect of antibody coating of the red cells, the effect of splenectomy in these patients, and the effects of adrenocortical steroid therapy.

## METHODS

**Patients.** 30 patients with autoimmune hemolytic anemia, warm antibody type, in whom the direct anti-globulin test (Coombs test) was positive using anti-IgG antiserum, and in whom the amount of IgG on the cells could be quantitated by the methods described below, were studied. A summary of the clinical course and laboratory data of these patients mentioned specifically in the text are given in Table I. The antibodies present in serum or eluates did not have specificity for known red cell antigens when tested on a panel of cells or when absorbed with cells of known Rh genotype.

**Blood samples.** Whole blood or washed red cells obtained after defibrination were mixed with one-quarter volume of Alsever's solution (11). Since preliminary tests indicated that the amount of antibody on the red cell surface decreased during storage, samples were analyzed no later than 24 hr after being drawn. Serum was obtained either by removing the serum after defibrination or by allowing the clot to retract at room temperature for 1-2 hr. The serum was heated at 56°C for 30 min and stored at -20°C until use.

**Buffer.** Veronal-buffered saline (VBS), pH 7.4, was made according to the method of Mayer (11). When buffers of reduced ionic strength were required, VBS was mixed with isotonic sucrose buffer, according to the directions given in reference 12.

**Antiserum.** Rabbit antiserum to human immunoglobulin G (IgG) was obtained by repeated injection of rabbits with purified IgG from patients with multiple myeloma. In some instances, anti-whole serum obtained from Ortho Pharmaceutical Corp., Raritan, N. J. was used. The anti-IgG molecules were IgG immunoglobulins.

**The  $\bar{C}_I$  fixation and transfer test.** The  $\bar{C}_I$  fixation and transfer test was performed according to the methods given in references 2 and 9. In order to test the amount of cell-bound antibody, the cells of the patient were washed three times in VBS. 0.25 ml of packed cells was suspended in 9.0 ml of 60% VBS-sucrose and the suspension was adjusted so that a 1:25 dilution had an optical density of 0.210 at 541  $m\mu$ . This standard suspension contains approximately  $2.2 \times 10^8$  red blood cells per ml. 0.1 ml of this suspension was mixed with 0.2 ml of an appropriate dilution of rabbit anti-IgG and incubated for 30 min at 30°C. The dilution used was such that slope of the plot of the logarithm of amount of primary antibody against the logarithm of the amount of  $\bar{C}_I$  fixed was equal to 1 (see references 9 and 13). The cell suspension was washed twice with 8 ml of 60% VBS-sucrose at about 500 g. After the pellet had been thoroughly dispersed with a vortex mixer, 0.3 ml of  $\bar{C}_I$ , which had been adsorbed twice with a  $\frac{1}{2}$  volume of packed human red cells, was added and the mixture was incubated at 30°C for 30 min. After incubation, the cells were washed, transferred to a new set of tubes, and washed three further times with 8 ml of 60% VBS-sucrose at each washing. After dilution in VBS, the amount of  $\bar{C}_I$  fixed was determined as outlined.

In order to determine the concentration of antibody in serum or eluate solutions, normal cells from a single O<sup>+</sup> donor were washed and a standard suspension was made. 0.1 ml of cells was mixed with 0.2 ml of serum and the mixture was incubated for 30 min at 30°C. The cells were washed twice with 60% VBS-sucrose and 0.2 ml in appropriate dilution (see above) of rabbit anti-IgG was added. After incubation for 30 min at 30°C, 0.3 ml of  $\bar{C}_I$  was added and the cells were further treated as in the quantitative direct Coombs test. The results were expressed as molecules  $\bar{C}_I$  fixed per red cell. The constraints upon this method of determining antibody in solution are given in references 2 and 9.

When papainized cells were required for the quantitative indirect Coombs test, they were prepared by incubating equal volumes of a standard suspension of cells with 1% cysteine-activated papain, at pH 7.4 for 20 min. After incubation, the cells were washed twice and resuspended in the original volume of 60% VBS-sucrose buffer.

**Red cell survival time.** Red cell survival time was measured by the endogenous production of carbon monoxide, by use of a gas phase analytic system described by Logue et al.<sup>1</sup> The red cell life span estimated from carbon monoxide was calibrated with the red cell life span determined by the diisopropylfluorophosphate (DFP<sup>SM</sup>) method of Cline and Berlin (14). The values expressed are for the corrected mean red cell life span; normal is 100-130 days.

## RESULTS

**Relationship between hemolytic rate and cell-bound antibody.** The amount of cell-bound antibody was determined in 25 patients with immune hemolytic anemia, warm antibody type, before treatment with steroids, splenectomy, or immunosuppressive agents. The relation-

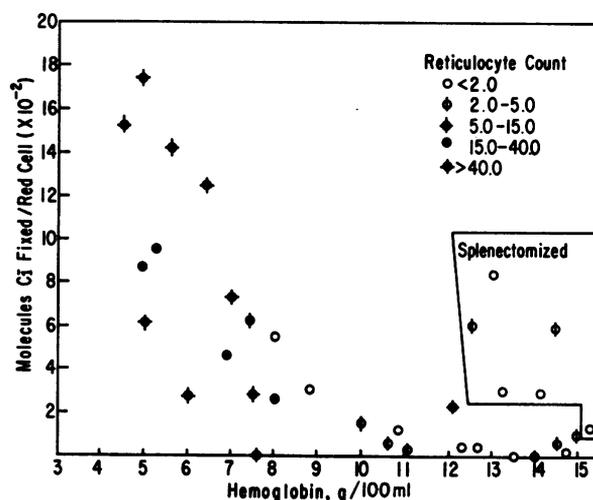


FIGURE 1 The relationship between the hemoglobin concentration in peripheral blood and the amount of antibody on the circulating red blood cells as measured by  $\bar{C}_I$  fixation in the presence of anti-IgG antibody. None of the patients were receiving adrenocorticosteroid or immunosuppressive therapy at the time of the test. The results from patients who had undergone splenectomy are shown in the boxed area.

TABLE I  
Brief Clinical Summary of Patients with Immune Hemolytic Anemia\*

Patient	Sex	Age yr	Onset of hemolytic disease			Direct Coombs			Treatment	Result†	Comments
			Underlying disease	Hemoglobin g/100 ml	Reticulocyte count %	Anti-IgG	Anti-C3	Treatment			
R. A.	F	66	CLL‡	4.6	1.0	+	+	Prednisone Chlorambucil	Partial remission	Died of sepsis	
E. B.	F	63	SLE	3.8	52.0	+	+	Prednisone	Remission		
B. B.	M	36		6.0	52.0	+	-	Prednisone	Complete remission		
J. B.	M	62	CLL	5.5	17.0	+	-	Prednisone Chlorambucil	Remission		
B. C.	M	62	CLL	6.2	15.6	+	-	Prednisone Chlorambucil	Remission		
R. F.	F	58	SLE	4.2	40	+	+	Prednisone Splenectomy Cytosax	Remission with exacerbation	SLE diagnosed 9 yr after onset of hemolysis	
C. F.	F	2		6.3	15	+	-	Prednisone	Remission	Died of demyelinating disease	
A. G.	F	22	Thalassemia intermedia	5.6	14.2	+	-	Prednisone Splenectomy	Remission	Exacerbation with viral illness	
M. G.	F	46	ITP¶	10.8	5.2	+	-	Cytosax	Remission		
C. H.	F	71		5.0	67.8	+	-	Splenectomy Prednisone	No remission	Died of serum hepatitis	
E. K.	F	60	CLL	3.3	1.8	+	-	Prednisone Chlorambucil	Remission		
S. M.	F		SLE	5.4	6.4	+	-	Prednisone	Remission		
M. P.	F	35		6.1	22.0	+	+	Prednisone Splenectomy Imuran	Complete remission	Systemic nocardiosis (cured)	
P. P.	M	52		6.8	20.0	+	-	Splenectomy	Complete remission		
G. R.	F	77	CLL	5.0	10.6	+	-	Prednisone Chlorambucil Cytosax	Remission		
D. S.	M	24	Infectious mononucleosis	5.2	27	+	-	Prednisone Splenectomy Cytosax	Remission	Exacerbation with "viral" diseases	
M. S.	F	57	CLL			+	-	Prednisone	Remission		
J. T.	M	2	Wiskott-Aldrich syndrome	4.0	32	+	+	Splenectomy 6-Mercaptopurine	No remission	Died of cytomegalovirus disease	
L. W.	M	56	"Viral" disease	10.3	9.3	+	+	Splenectomy Prednisone	Lost to follow-up		
M. W.	F	62	Malabsorption syndrome	4.3	2.3	+	-	Prednisone	Remission		

\* Data shown only on patients specifically mentioned in text.

† Complete remission = normal blood with no therapy; remission = normal hemoglobin with less than 10 mg of prednisone; partial remission = normal hemoglobin with more than 10 mg of prednisone; no remission = not able to maintain normal hemoglobin.

‡ Chronic lymphocytic leukemia.

|| Systemic lupus erythematosus.

¶ Idiopathic thrombocytopenic purpura.

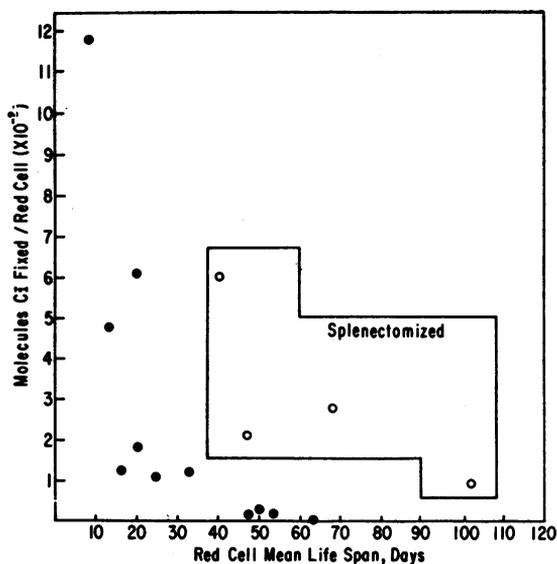


FIGURE 2 The relationship between mean red cell life span, measured by the endogenous production of carbon monoxide, and the amount of antibody on the circulating red blood cells, as measured by fixation of C<sub>1</sub> in the presence of anti-IgG antibody. The results for splenuctomized patients are shown in the boxed area.

ship of the amount of antibody on the cell to the hemolytic rate, as judged by either the decrease in hemoglobin and increase in reticulocyte count or by the production of carbon monoxide is shown in Figs. 1 and 2. Although small amounts of cell-bound antibody, in some instances not readily detectable by this assay, appear to reduce the red cell life span as measured by endogenous

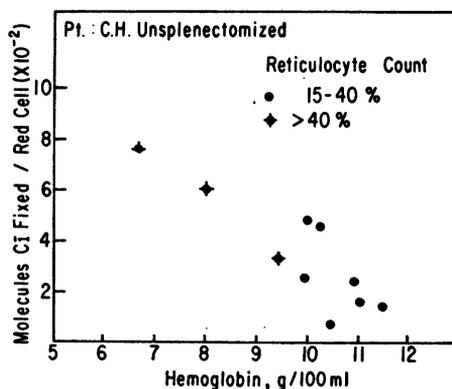


FIGURE 3 The relationship between hemoglobin concentration in the peripheral blood and concentration of cell-bound antibody in patient C. H. during adrenocorticosteroid therapy.

carbon monoxide production, marked reduction in the life span and consequent anemia occurs when large amounts of antibody are present on the cell. There is considerable variation from patient to patient in the amount of cell-bound antibody which effects a given amount of hemolysis or diminution in hemoglobin concentration.

In individual patients, the relationship between the amount of cell-bound antibody and the rate of hemolysis or reduction in hemoglobin is more clearly shown (Fig. 3). In eight patients, the amount of cell-bound antibody was measured before and after the onset of a hemolytic exacerbation (Table II). These data indicate that the main determinant for the rate of destruction of red cells in warm antibody hemolytic anemia is the amount of cell-bound antibody.

TABLE II  
Changes in Concentration of Cell-Bound Antibody during Exacerbation of Immune Hemolytic Anemia

Patient	Therapy before exacerbation	Date	Hemoglobin	Reticulocyte count	Cell-bound antibody	Serum antibody
			g/100 ml	%		
M. P.	Steroids*	12-9-66	9.1	13.0	60	80
		12-12-66	6.8	26.0	1590	1020
G. R.	Steroids Chlorambucil	8-2-68	10.2	2.3	23	100
		9-20-68	5.0	10.6	610	590
B. B.		11-3-66	14.2	5.8	0	80
		12-1-66	10.5	16.6	210	580
C. F.		5-17-67	12.5	0.8	30	205
		10-6-67	6.8	23.0	460	410
R. F.	Steroids Splenuctomy	7-25-66	9.0	5.0	625	1000
		7-31-66	4.8	23.0	2500	250
A. G.	Steroids Splenuctomy	12-1-67	9.8	4.3	200	
		12-26-67	4.5	48.0	2260	

\* Usually prednisone.

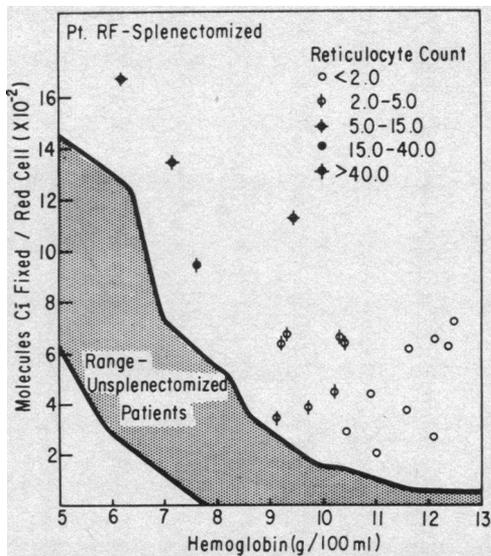


FIGURE 4 The relationship between hemoglobin concentration in the peripheral blood and the concentration of cell-bound antibody in patient R. F. who had undergone splenectomy. The range for unsplenectomized patients (taken from Fig. 1) is shown in the shaded area. The patient was taking 0-10 mg of prednisone at the time of the determinations.

*The effect of splenectomy.* The relationship between the amount of cell-bound antibody and the hemolytic rate as judged by endogenous carbon monoxide production or reduction in hemoglobin was determined in six patients who had been splenectomized (Figs. 1 and 2). These patients appeared to have considerably less hemolysis for a given concentration of cell-bound antibody than pa-

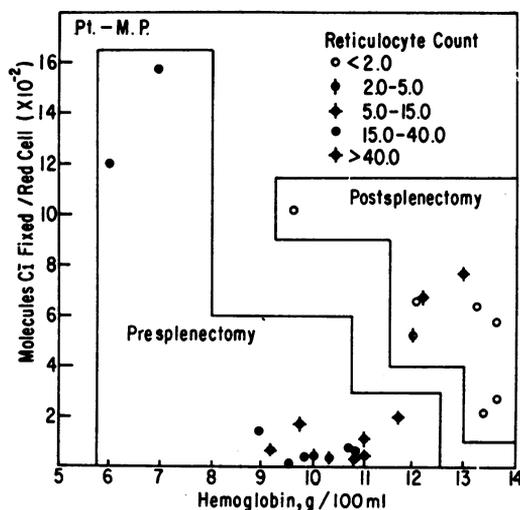


FIGURE 5 The relationship between the concentration of hemoglobin in peripheral blood and the concentration of cell-bound antibody in patient M. P. before and after splenectomy.

tients who had not been splenectomized. However, hemolysis occurred when the amount of cell-bound antibody became excessive (Fig. 4, Table II, patients A. G. and R. F.). One patient, M. P., was extensively studied before and after splenectomy (Fig. 5). The increased tolerance for cell-bound antibody after splenectomy is clearly shown.

In five patients, the amount of antibody attached to red cells extruded from the spleen at operation was compared to the amount present in the peripheral blood on the same day (Table III). In each case, the amount of antibody on the red cells from splenic blood exceeded that present on the red cells in the peripheral blood.

*The effect of prednisone therapy.* 15 patients were given 60 mg of prednisone per day at the beginning of therapy. In 10 patients, the amount of antibody present on the cells fell rapidly. In eight of these patients, this rapid diminution of the cell-bound antibody was accompanied by an increase in the amount of antibody present in the serum. When tested, the mean red cell life span was seen to increase as the amount of antibody decreased (see Figs. 6 and 7). This effect appears to be a function of the high dose of prednisone given since, in patient R. A. at day 20, when the dosage of prednisone was reduced from 60 mg/day, the amount of antibody present on the cell began to increase and the amount of antibody in the serum to decrease. When the dose of prednisone was again raised to 60 mg/day on day 25, the cell-bound antibody again decreased (see Fig. 7).

In five patients, the rapid removal of cell-bound antibody was not accompanied by an increase in the antibody cell-bound in the serum (Fig. 8). In three patients, the amount of cell-bound antibody diminished only slowly over a period of 10-20 days. In one patient, prednisone appeared to be virtually without effect on the concentration of cell-bound antibody. This patient did not have a remission in hemolytic rate as judged by a decreased hematocrit and continued transfusion requirement.

TABLE III  
Cell-Bound Antibody on Peripheral Red Cells and on Red Cells Removed from the Spleen after Splenectomy

Patient	Molecules of CI fixed per red cell		Mean red cell life span	
	Peripheral blood	Splenic blood	Before splenectomy	After splenectomy
	<i>days</i>			
D. S.	130	195	16	48
P. P.	47	160		103
M. P.	70	90		64
M. S.	90	1110	28	78
M. G.	30	115	40	82
M. W.*	5	0	30	108

\* Patient with hereditary spherocytosis.

A second effect of prednisone is a diminution in the amount of antibody present in the serum (Table IV). This effect was seen in all patients undergoing clinical remission with prednisone treatment. The time required for the concentration in serum antibody to reach low levels ranged from 4 to 87 days. This time period appeared to be longer for patients with chronic lymphocytic leukemia than for patients not having this disease.

Once the amount of antibody present in the serum had fallen, prednisone dosage could be safely reduced to levels of less than 10 mg/100 ml in all patients achieving this response. Three patients did not have a marked diminution in the amount of antibody present in the serum. In these patients, the amount of antibody on the cells, the amount of antibody in the serum, and the hemolytic rate remained high.

A third effect of prednisone was deduced from analysis of the results of patient R. A. (see Fig. 6). In this patient, the amount of cell-bound antibody present on day 20 was approximately that present before the onset of prednisone therapy. However, the mean red cell life span was approximately four times the initial value. This suggests that prednisone may interfere with the sequestration of the antibody-coated red cells by the reticuloendothelial system.

## DISCUSSION

The red cells of patients with autoimmune hemolytic anemia of the warm antibody type are randomly destroyed as the result of the immunological reactions which take place on their surface. In some instances, complement is fixed. However, in many, if not most instances complement is not fixed efficiently since two molecules of the IgG antibody are needed for the fixation of a single molecule of the first component of complement. Therefore, the greatest proportion of the hemolysis which occurs in these patients is probably the result of the presence of antibody on the cell surface.

The quantitative relationship between the amount of antibody present on the cell surface and the rate of destruction of the cells has been somewhat obscure in the past. Jandl and Kaplan showed D-positive red cells sensitized with anti-D and injected into normal recipients were rapidly destroyed (15). The rate of destruction was roughly parallel to the amount of antibody used in sensitization. These findings were confirmed and expanded by the studies of Mollison, Crome, Hughes-Jones, and Rochna (5). These investigators used much higher concentrations of antibody and observed much more rapid rates of red cell destruction.

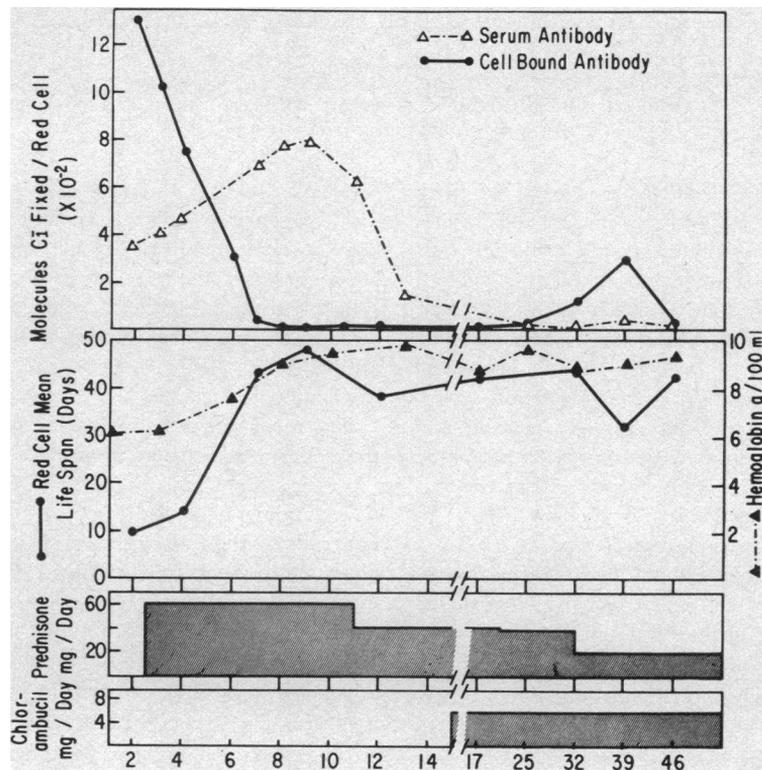


FIGURE 6 The changes in cell-bound and serum antibody concentration, hemoglobin concentration in the peripheral blood, and mean red cell life span after prednisone therapy in patient B. C.

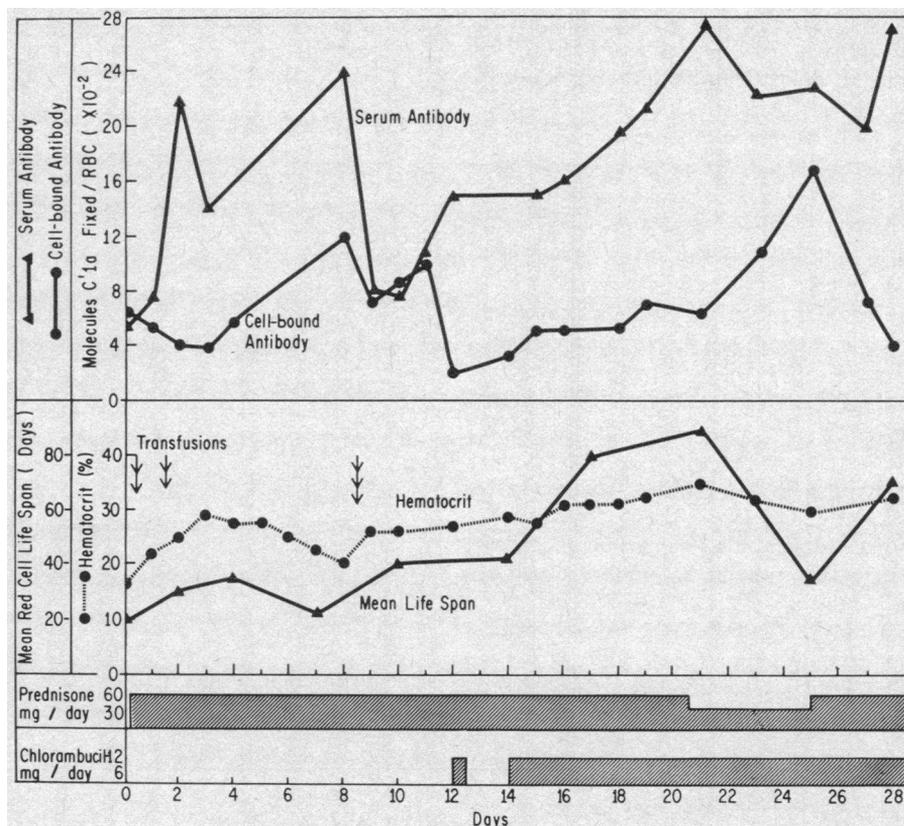


FIGURE 7 The changes in cell-bound and serum antibody concentration, hematocrit, and mean red cell life span after prednisone therapy in patient R. A.

Constantoulakis, Costea, Schwartz, and Dameshek measured the amount of antibody on the red cells of patients with autoimmune hemolytic anemia using an anti-IgG labeled with <sup>125</sup>I and sought to relate the level of antibody to the red cell life span as measured by <sup>51</sup>Cr (6). Although they could demonstrate with a single example of anti-D that the amount of antibody on cells sensitized in vitro was related to their life span, they found no relationship between the amount of antibody coating and destruction rate in patients with immune hemolytic anemia. They attributed this lack of correlation to variability in the avidity of antibody for the red cell or to factors other than concentration of antibody.

The present studies suggest that the concentration of antibody bound to the red cell is, in fact, a prime determinant of the red cell survival time in these patients. Although there was considerable variation among the patients studied, those who were most anemic at presentation and/or who had the greatest production of endogenous carbon monoxide as a measure of heme destruction were those whose red cells were most heavily coated with antibody. This appears to be so whether or not the antibody was capable of fixing complement. In in-

dividual patients studied serially, the red cell life span was, in general, inversely related to the amount of antibody present on the red cell. When hematologic relapse occurred, the concentration of antibody on the cells was invariably increased above levels present during remission, and when remission occurred, the amount of antibody was lower in nearly all instances than during the hemolytic episode.

The mechanism by which the presence of antibody on the cell surface brings about the destruction of the cell is not at all certain. Archer (3) and Lo Buglio, Cotran, and Jandl (4) found that antibody-coated cells were adherent to the monocytes or splenic macrophages. This appeared to result in engulfment or loss on the part of the membrane resulting in spherocytes. This adherence phenomenon appears to be a function of the IgG molecule since none of the other immunoglobulins or complement appear to be able to effect this reaction. This phenomenon appears to be different from erythrophagocytosis since polymorphonuclear leukocytes cannot engulf IgG antibody-coated cells in the absence of complement (16). From the present studies, it appears that the greater the number of IgG molecules coating the cell, the greater

likelihood that the cells will adhere to macrophages of the reticuloendothelial system.

The data on cells extruded from the spleen might indicate that more heavily sensitized cells were sequestered preferentially by the spleen. However, the increase in cell-bound antibody on these cells might also be due to local production of antibody in splenic cells.

The spleen is of paramount importance in the process of destruction of the red cells in patients with IgG warm agglutinin disease. Removal of the spleen reduces the rate of hemolysis in many patients. The data from the patients studied here suggests that when the spleen is removed, up to 10 times as much antibody must be present on the red cell to effect the same degree of lysis. This would suggest that the other sites of attachment of IgG-coated cells within the reticuloendothelial system were less efficient than splenic sites. When, however, the concentration of antibody on the cell reaches a sufficient concentration, sequestration does occur and the hemolysis results, despite the absence of the spleen.

These findings corroborate single studies by Constakoulakis et al. (6) and by Jandl and Kaplan (15). The former group found that cells from a patient with immune hemolytic anemia survived longer in a splenectomized patient than in an unsplenectomized patient. Jandl and Kaplan found that more antibody had to be added to cells in vitro in order to obtain the same rate of red cell destruction in a splenectomized patient compared to an unsplenectomized patient.

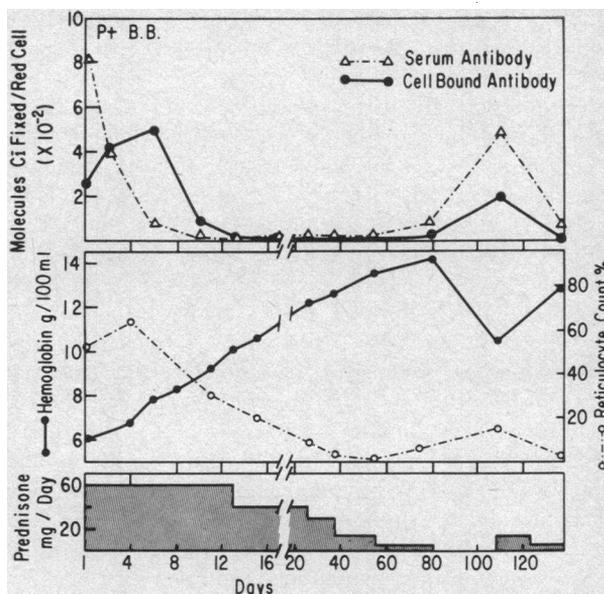


FIGURE 8 The changes in cell-bound and serum antibody concentration, hemoglobin concentration, and reticulocyte count after prednisone therapy in patient B. B.

The adrenocortical steroids have been known for at least 20 yr to induce remission in patients with autoimmune hemolytic anemia of the warm antibody type (17). Three mechanisms were proposed for the effect: (a) diminution of antibody production (18), (b) diminution of sequestration (19), and (c) alteration of the red cell-

TABLE IV  
Changes in Cell-Bound and Serum Antibody after High Dose Prednisone Treatment (60 mg/day) in Patients with Immune Hemolytic Anemia

Patient	Cell-bound antibody, molecules of CI/cell	No. of days until cell-bound antibody 100 molecules CI or less	Increased antibody in serum	Initial serum antibody, molecules CI/cell	No. of days to 100 molecules of CI or less
R. F.	2500	16	Yes	250	32
G. R.*	610	5	Yes	584	87
L. R.	340	5	Yes	490	> 14
M. W.	80	—	Yes	225	
C. F.	470	3	Yes	220	9
M. P.	1600	6	Yes	150	9
B. C.*	1300	5	Yes	350	12
R. A.*	600	> 28	Yes	550	> 28
S. M.	116	20	No	529	?
B. B.	225	10	No	820	4
E. B.	1510	3	No	330	4
J. B.*	610	10	No	450	24
E. K.*	570	4	No	260	30
C. H.	260	> 213‡	No	280	> 213‡
J. T.	970	> 92‡	No	800	> 92‡

\* Patients with prior chronic lymphocytic leukemia.

‡ Remission as defined by these parameters was not achieved during the lifetime of the patient.

antibody relationship (20). Without the quantitative methods used in these studies, it has been very difficult to test these hypotheses in patients. Certainly, in experimental animals, it is possible to demonstrate diminution of antibody production after treatment with steroids, especially IgG antibody of the secondary immune reaction (18, 21, 22). Using dilution techniques, Evans, Bingham, and Boehni, among others, have found that the amount of serum antibody decreased during prednisone therapy in patients with autoimmune hemolytic anemia (18). The present studies demonstrate that the total amount of antibody (cell bound + serum) is usually decreased after prednisone therapy. The time interval between the initiation of prednisone therapy and the reduction in serum antibody varies from 4 to 90 days. This interval tended to be longer in patients with chronic lymphocytic leukemia and reduction in serum antibody sometimes did not occur until the leukemic process was brought under control by other chemotherapeutic agents. If the reduction in serum antibody did not occur, remission was difficult to obtain and maintain. This reduction in serum antibody, when it did occur, could usually be maintained by relatively small doses of prednisone (10 mg or less per day). If the dose was reduced too far, the amount of antibody in the serum rose as did the amount of cell-bound antibody and increased hemolysis occurred (see Fig. 7). Clearly the diminution in total concentration (serum + cell bound) is probably the most important effect of prednisone; how it is brought about is unknown.

An effect of prednisone in decreasing sequestration may also be inferred from these data. In patient R. A., the mean red cell life span was longer for the same or greater doses of cell-bound antibody when prednisone was given than before the initiation of that therapy. This effect of prednisone has been postulated by Kaplan and Jandl in studies on rats injected with antibody-coated cells (19). They found that prednisone decreased the sequestration of cells in the reticuloendothelial system other than the spleen. The present data do not allow determination of the importance of this effect of steroids nor localization of the effect to any part of the reticuloendothelial system.

Prednisone may alter the affinity of antibody for the red cell. In more than half the patients studied, the amount of antibody on the cells fell rapidly and the amount in the serum rose upon the initiation of high doses of the drug. Where measured, the red cell survival increased in parallel as the amount of cell-bound antibody decreased. The rise in serum antibody might likewise be explained by sudden release of stored antibody from lymphocytes or other cells. This effect of prednisone appears to be seen only with high doses of the

drug and probably accounts for the rapid, early amelioration of hemolysis after the institution of therapy. The basis of this effect is the subject for further investigation.

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