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Research Article

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Corticosteroid therapy increased the survival of both IgG and IgM sensitized erythrocytes by decreasing sequestration in the reticuloendothelial system (RES). 5 days of therapy prior to injection of antibody coated cells were required for a maximal effect. It appeared that the RES of cortisone-treated animals had a lowered sensitivity to erythrocytes coated with antibody and complement and the cells were removed as though they were coated with fewer complement sites/cell. The general pattern and kinetics of clearance and the localization of sequestered cells were not modified by corticosteroids. As the number of IgG C1-fixing sites was increased, the difference between cortisone treated and control animals was less marked.

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ABSTRACT Corticosteroids and splenectomy constitute two important therapeutic modalities in the treatment of autoimmune hemolytic anemia. Each of these may affect both the rate of synthesis of autoantibody and the clearance of antibody sensitized cells. The latter possibility has been examined in an experimental model which allows evaluation of the role of antibody and complement in the immune clearance of erythrocytes in molecular terms. The *in vivo* clearance of ⁵¹Cr-labeled guinea pig erythrocytes sensitized with purified rabbit IgG or IgM antibody to produce a known number of complement-fixing sites per cell was studied.

Corticosteroid therapy increased the survival of both IgG and IgM sensitized erythrocytes by decreasing sequestration in the reticuloendothelial system (RES). 5 days of therapy prior to injection of antibody coated cells were required for a maximal effect. It appeared that the RES of cortisone-treated animals had a lowered sensitivity to erythrocytes coated with antibody and complement and the cells were removed as though they were coated with fewer complement sites/cell. The general pattern and kinetics of clearance and the localization of sequestered cells were not modified by corticosteroids. As the number of IgG C1-fixing sites was increased, the difference between cortisone treated and control animals was less marked.

Splenectomy led to an increased survival of IgG-coated cells and a sixfold increase in IgG C1-fixing sites was necessary in order to obtain similar rates of clearance in splenectomized and control animals. The liver was responsible for this much less efficient clearance of cells in splenectomized animals; the clearance pattern was typical of that noted for IgG. No effect at all was noted on the clearance of IgM sensitized cells in splenectomized animals.

These experiments clearly demonstrate that both corticosteroid therapy and splenectomy act to decrease the *in vivo* clearance of IgG-sensitized cells; only corticosteroids alter the clearance of IgM-sensitized erythrocytes. This effect may be of major importance in explaining the efficacy of these therapeutic modalities in autoimmune hemolytic anemia.

INTRODUCTION

Despite the lack of a clear understanding of the etiology and pathophysiologic basis of autoimmune hemolytic anemia (AIHA),¹ effective therapy has been developed. High dose corticosteroid therapy will induce a complete or partial remission in most patients with this disease. Some of the patients who respond poorly to corticosteroid therapy can be effectively treated with splenectomy (1, 2). The mode of action of these two therapeutic modalities has been difficult to define in patients since each of these methods of therapy may affect both the level of sensitization of erythrocytes with autoantibody and the rate of clearance of sensitized cells. Steroid therapy may reduce sensitization of antibody coated erythrocytes by decreasing antibody production or by decreasing the binding of antibody to the red cell surface (3, 4). On the other hand, corticosteroids are known to influence the function of the reticuloendothelial system (5, 6). Similarly, the spleen can be an important site of antibody synthesis (7) and splenectomy may therefore lead to decreased levels of erythrocyte sensitization. The spleen is also known to be a major site for the sequestration of antibody-coated erythrocytes and the effect of splenectomy may be to decrease clearance of these cells.

In previous papers (8, 9) we have described an experimental model of immune hemolytic anemia designed to examine the role of complement-fixing IgG and IgM antibody and of complement in the immune clearance and

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¹Abbreviations used in this paper: AIHA, autoimmune hemolytic anemia; RES, reticuloendothelial system; VBS, veronal-buffered saline.

destruction of erythrocytes. In this model, every IgM antibody molecule in a rabbit anti-guinea pig erythrocyte preparation fixed complement and at least 60 complement-fixing sites per erythrocyte were required for accelerated clearance of IgM-sensitized cells. The pattern of clearance of IgM coated erythrocytes was that of rapid sequestration in the liver and then slow return to the circulation as Coombs positive cells. In contrast, approximately 2,000 IgG antibody molecules were required to form a complement-fixing site. However, as few as 1.4 complement-fixing sites per erythrocyte resulted in decreased survival. The clearance pattern for IgG-sensitized cells was that of progressive trapping by the spleen. Clearance of IgM-coated cells was absolutely complement dependent and that of IgG was largely complement dependent, although IgG immune globulin coating alone could affect clearance rates. In this paper we extend this model to explore in molecular terms the effects of corticosteroids and splenectomy on the clearance of IgG- and IgM-sensitized cells.

METHODS

The preparation and source of buffers, complement reagents, guinea pig erythrocytes and rabbit anti-guinea pig erythrocyte antisera were previously described, as were methods for immunoglobulin purification, erythrocyte survival, and quantitative antibody and complement studies (8-10).

Drugs. Cortisone acetate 25 mg/cc (The Upjohn Co., Kalamazoo, Mich.) was administered subcutaneously. Hydrocortisone sodium succinate (Solu-cortef, The Upjohn Co.) was administered intraperitoneally or in *in vitro* studies added directly in 0.1 ml portions to veronal-buffered saline (VBS).

Clearance studies. The techniques used in clearance studies have been described in detail (8, 9). In brief, ^{51}Cr -labeled normal guinea pig erythrocytes were coated with highly purified, high avidity rabbit IgG or IgM antibodies to produce a known number of complement fixing sites as determined by the C1a fixation and transfer test. A volume of 1 ml containing 2.7×10^8 sensitized cells or control unsensitized cells was injected into the hind-foot vein of the guinea pigs and erythrocyte survival was determined by serial 0.1 ml bleedings from the retroorbital sinus using a calibrated bleeding pipette. The blood samples were suspended in VBS containing 0.01 M ethylenedinitrilotetraacetic acid and the number of counts per milliliter of blood determined utilizing a gamma scintillation counter. Treatment and control groups were always studied simultaneously and freshly prepared cells were used in clearance studies within 2 h after sensitization. Appropriate controls to rule out significant agglutination and intravascular hemolysis were performed. Localization of antibody-coated erythrocytes was determined at several levels of sensitization in splenectomized and cortisone-treated guinea pigs. Groups of animals were sacrificed at 2 h; the liver, lungs, spleen, and kidney were removed and radioactivity of the entire organ was determined. The clearance data were analyzed and plotted as previously described (8). Means were compared with Student's *t* test and the results expressed as *P* values (levels of significance).

In vitro studies with hydrocortisone. Studies were performed to determine whether hydrocortisone sodium succinate might alter binding of antibody to the erythrocyte surface. As a model for what might occur *in vivo*, erythrocytes prepared up to the point of sensitization were incubated for 30 min at 37°C in VBS containing 100 µg/ml hydrocortisone sodium succinate (cortisone buffer). These erythrocytes were then sensitized with antibody diluted in cortisone buffer, washed and incubated in this buffer for periods of 30, 60, or 90 min at 37°C. Following incubation, the cells were washed in VBS and resuspended to the original volume (2.7×10^8 erythrocytes/ml). Clearance studies were then performed with these cells and erythrocytes sensitized in and incubated in VBS only as a control.

Effect of cortisone acetate on the hematocrit, body weight, and spleen and liver weights. Two groups of guinea pigs were administered daily injections of either 20 mg/kg or 100 mg/kg of cortisone acetate. A third group was given an equivalent volume of sterile saline as a control. Groups were maintained under similar conditions of husbandry and weighed every 2nd day. Prior to sacrifice on the 14th day of the study, blood was obtained from the retroorbital venous plexus for determination of the hematocrit.

Splenectomy. Anesthesia was maintained during the procedure by one intraperitoneal injection of 200 mg/100 g body weight of ethyl urethane (Fisher Scientific Company, Fair Lawn, N. J.) and intermittent inhalation of ether (Mallinckrodt Chemical Works, St. Louis, Mo.). A 4 cm skin incision was made along the lower left rib margin and the incision carried through the peritoneum. The splenic vessels were tied with nylon and the spleen removed. The peritoneum and subcutaneous structures were closed with 4-0 chromic gut and the skin was closed with clamps. Antibiotics were not utilized. After a postoperative period of 1 wk or more, splenectomized animals were used for clearance studies if the hematocrit was within the normal range and the animal was gaining weight. A sham-operated group of animals underwent a similar series of surgical manipulations but was not splenectomized.

RESULTS

Time required for cortisone acetate therapy to alter the rate of clearance of radiolabeled erythrocytes sensitized with 17 IgG C1-fixing sites per cell (Table 1). Animals administered cortisone acetate for 1, 2, and 3 days prior to clearance studies had clearance patterns which were identical to those of untreated guinea pigs. However, animals studied on the 4th day of treatment with cortisone acetate had a significantly higher percentage of sensitized cells remaining in the circulation at 2 h than control groups ($P < 0.05$). By the 5th day of treatment this difference had become maximal and did not significantly vary during the course of the next 12 days of treatment. In all subsequent studies animals received cortisone acetate for at least 7 days prior to study. This moderate level of erythrocyte sensitization was arbitrarily chosen. Similar but more limited studies with 117 IgM sites per cell and 8 and 34 IgG sites per cell gave similar results.

The effect of cortisone acetate on the survival of IgM-sensitized erythrocytes. In normal, untreated guinea

TABLE I
Time Required for Cortisone Acetate Therapy to Increase the Survival of IgG-Sensitized Erythrocytes

Days of therapy prior to study	Mean percent of radiolabeled erythrocytes sensitized with 17 IgG C1-fixing sites remaining in the circulation after 2 h			
	Mean percent	Range	Standard error	No. of animals
0	4.5	0.7-10.7	0.66	15
0.2*	6.1	1.0-10.2	1.7	6
1	3.8	1.7- 9.5	1.4	5
2	3.7	2.2- 5.3	1.5	2
3	4.2	3.8- 4.5	0.35	3
4	13.2‡	3.7-20	4.9	3
5	23.6§	3.5-41	8.2	4
6-17	26.3§	8.0-55	5.1	10

* Animals received 100 mg/kg of intraperitoneal hydrocortisone sodium succinate 4 and 2 h before study. Subsequently, in the above study animals were administered either 20 or 100 mg/kg per day of cortisone acetate. ‡ $P < 0.05$ when compared with days 0-3. § $P < 0.01$ when means compared with days 0-3 and $P < 0.05$ when means compared with day 4.

pigs, injection of erythrocytes with 117 IgM C1-fixing sites per cell was followed by the clearance of between 50 and 75% (mean 62%) of the injected cells by the liver within 10 min. Over the next 2 h, approximately one-third of these cells were returned to the circulation. In initial studies animals were treated with a single injection of 10 mg/kg per day of cortisone acetate. This dose was chosen because it is reported to suppress reticuloendothelial function in many species (6). Treatment with this regimen reduced the percent of cells removed by the liver within the first 10 min to a mean of 45 (Fig. 1). Increasing the cortisone dose to 20 or 100 mg/kg per day further decreased sequestration and the variation from animal to animal was less. However, the further reduction in sequestration was not striking considering the magnitude of the increase in therapy. Studies of the organ localization of injected cells confirmed the findings of clearance studies. Counts of organs revealed decreased liver sequestration in cortisone treated animals (Table II). There was no evidence of splenic sequestration of IgM-coated cells at autopsy in either untreated or cortisone-treated groups.

When untreated animals were injected with erythrocytes containing 234 IgM C1-fixing sites per cell, more than 75% of the cells were sequestered within 10 min and only about 20% of these erythrocytes were returned to the circulation. In animals treated with 20 mg/kg per day of cortisone acetate (Fig. 2), slightly less than 50% of the cells were cleared during the first 10 min. At 351 IgM C1-fixing sites per erythrocyte, cortisone acetate also significantly reduced the percent of cells cleared by the liver. At this high level of sensitization, no cells

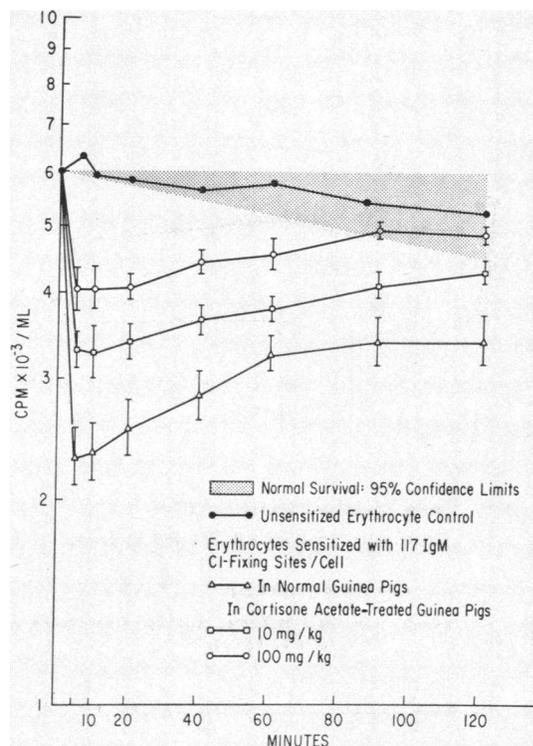


FIGURE 1 Survival of ^{51}Cr -labeled guinea pig erythrocytes sensitized with 117 IgM C1-fixing sites per cell: comparison between normal and corticosteroid-treated guinea pigs. These data represent the mean \pm standard error of 12, 8, and 6 animals in the control, 10 mg and 100 mg groups, respectively. The clearance curve in guinea pigs treated with 20 mg/kg per day of cortisone acetate fell approximately half-way between those for 10 and 100 mg. The shaded area in this and subsequent figures represents the 95% confidence limits for decay curve slopes of 20 normal animals.

TABLE II
*Organ Localization of Radiolabeled Sensitized Erythrocytes in Cortisone-Treated Compared with Untreated Guinea Pigs**

	Circulation	Liver	Spleen
Unsensitized control (15)‡	87.0 \pm 6.0	6.3 \pm 6.0	3.5 \pm 1.4
117 IgM C1-fixing sites			
Untreated (6)	34.2 \pm 3.2	62.3 \pm 3.7	3.7 \pm 1.6
Cortisone (4)§	48.4 \pm 2.1	46.9 \pm 4.8	4.1 \pm 1.8
3 IgG C1-fixing sites			
Untreated (4)	26.8 \pm 3.5	22.7 \pm 5.9	37.1 \pm 11.1
Cortisone (4)§	52.1 \pm 13.8	15.7 \pm 1.7	22.8 \pm 8.9
17 IgG C1-fixing sites			
Untreated (5)	4.5 \pm 0.7	23.9 \pm 9.9	69.1 \pm 1.0
Cortisone (5)§	24.3 \pm 6.2	22.3 \pm 5.7	47.5 \pm 5.3

* There was always less than 5% of the cells sequestered in the lungs and kidneys. No attempt has been made to correct for radioactivity of blood within organs.

‡ Number of animals in each group.

§ Cortisone acetate 10 mg/kg per day; 20 mg/kg per day for the IgG studies.

|| $P < 0.05$ when compared with the untreated group at the same level of sensitization.

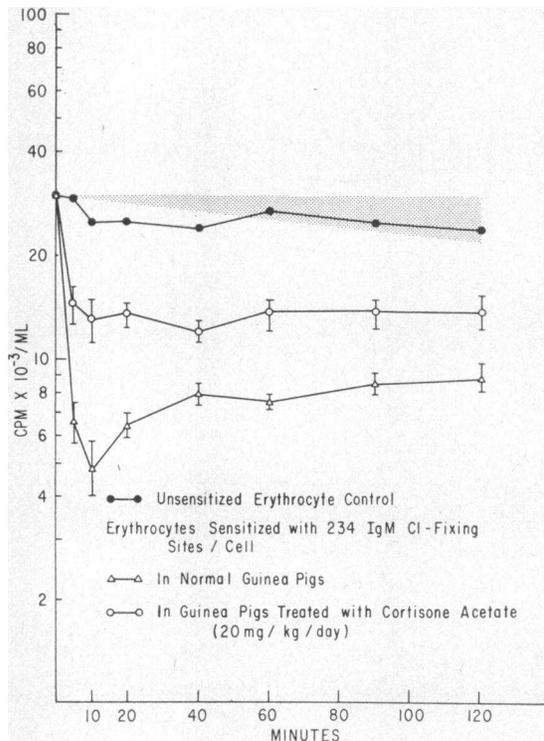


FIGURE 2 Survival of ^{51}Cr -labeled guinea pig erythrocytes sensitized with 234 IgM C1-fixing sites per cell: comparison between normal and corticosteroid-treated guinea pigs. Each point in these data represent the mean \pm standard error of eight animals in the control group and four in the corticosteroid-treated group. The clearance curves in animals treated with 10 or 100 mg/kg per day of cortisone acetate were not significantly higher than the sensitized control and 20 mg group, respectively.

were returned to the circulation in control or cortisone-treated guinea pigs. Treatment with corticosteroids did not appear to modify the kinetics of IgM clearance. Sensitized cells in cortisone-treated animals behaved as if they had fewer C1-fixing sites but the rates of clearance and release back into the circulation were unaltered.

The effect of cortisone acetate on the survival of IgG-sensitized erythrocytes. In normal guinea pigs injection of erythrocytes sensitized with 17 IgG C1-fixing sites per cell led to progressive erythrocyte sequestration (Fig. 3). At least 90% of the cells were removed from the circulation by 2 h. Of these, about 70% were sequestered in the spleen and about 25% were sequestered in the liver (Table II). Mean erythrocyte survival at 2 h was significantly increased ($P < 0.05$) by treatment with 10 mg/kg per day of cortisone acetate (Fig. 3). As with the IgM data, a wider variation from animal to animal was observed at low as compared with high cortisone dosages. In 20 or 100 mg/kg per day therapeutic groups, 27% of the cells remained in the circu-

lation to survive normally as compared with 16% at 10 mg and 6% for untreated controls. The pattern of sequestration of IgG-sensitized cells was unaltered by corticosteroid therapy but the cells behaved as if they were coated with fewer IgG C1-fixing sites per cell.

The effect of varying the level of erythrocyte sensitization was explored. When the number of C1-fixing sites was increased to 34 sites per cell, most of the protective effect of cortisone acetate was lost. Less than 3% of the cells remained in the circulation after 2 h even in the group of animals treated with 100 mg/kg per day of cortisone. At lower levels of sensitization, the cortisone effect was more pronounced. For example, at 7 and 3 (Fig. 4) IgG C1-fixing sites per cell, 10, 20, or 100 mg/kg per day of cortisone acetate therapy markedly decreased clearance. The data obtained with IgG-coated cells showed, therefore, a clear cortisone dose response relationship at low levels of sensitization. As the level of sensitization was increased the cortisone effect diminished. Thus, as one increases the level of sensitization, higher

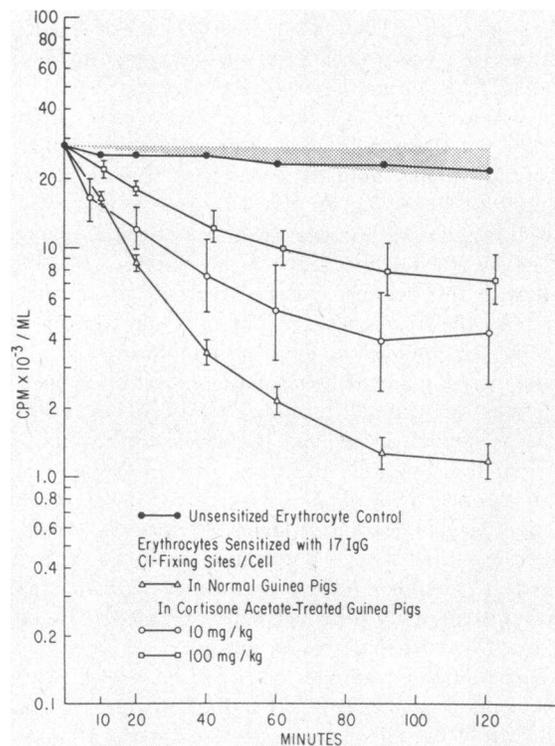


FIGURE 3 Survival of erythrocytes sensitized with 17 IgG C1-fixing sites per cell: comparison between normal and corticosteroid-treated guinea pigs. Each point in these clearance slopes represents the mean \pm standard error of 12, 10, and 4 animals in the untreated control, 10 mg and 100 mg groups, respectively. The clearance slope for the 20 mg group was not significantly different from the 100 mg group.

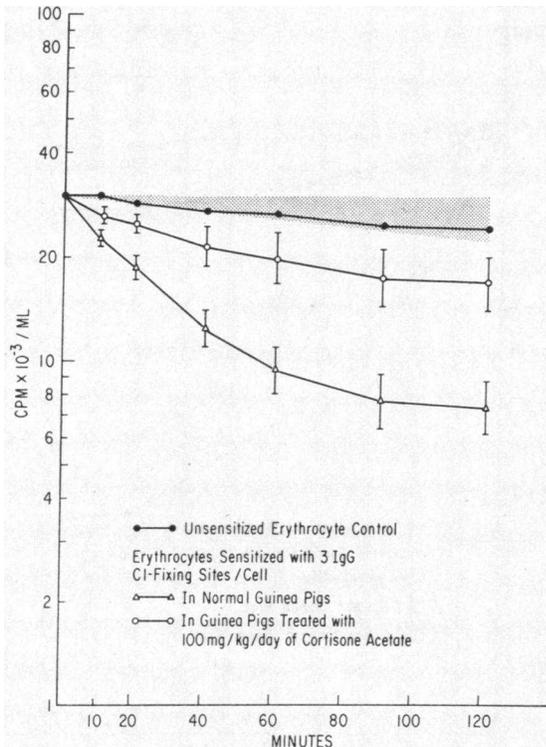


FIGURE 4 Survival of erythrocytes sensitized with 3 IgG C1-fixing sites per cell: comparison between normal and corticosteroid-treated guinea pigs. These data represent the mean \pm standard error of six animals at each point. The clearance curves for the 20 mg/kg per day group were not significantly different from the 100 mg group. 10 mg/kg per day dose significantly increased survival but not to the degree of the 20 or 100 mg groups.

and higher levels of corticosteroid are required for a therapeutic effect.

At all levels of sensitization progressive trapping of IgG-coated cells usually continued for about 90 min after injection and after this period cells survived normally even when corticosteroid therapy was discontinued (Fig. 5). This strikingly illustrates the fact that sensitized cells were only subject to clearance by the liver and spleen for a relatively brief period after injection. Thereafter they survived normally. The only exception was noted with very few (i.e. 3) IgG C1-fixing sites per cell in normal and treated guinea pigs where clearance occasionally would continue for up to 6 h. In no case did cortisone treatment appear to prolong the period of clearance. As shown in Table II, the increase in the number of cells remaining in the circulation at varying levels of sensitization could be accounted for by decreased splenic and/or hepatic sequestration.

Survival of IgM and IgG sensitized erythrocytes in splenectomized animals. Splenectomy had no effect on the rate of clearance of IgM-coated erythrocytes. This

was true at all levels of sensitization studied. Similarly, clearance patterns in sham-operated animals were like those in normal controls.

At 3 and 8 IgG C1-fixing sites per cell, 70 and 83% of cells were sequestered, respectively, in 2 h in normal guinea pigs. In animals following splenectomy, less than 20% of the cells were cleared at either of these two levels of sensitization. In fact, at these low levels of sensitization, clearance patterns in some of the animals were identical to those obtained with unsensitized cells. As shown in the bar graph (Fig. 6), clearance increased as the level of sensitization was increased until, at a level of 90 sites/cell, the effect of splenectomy was lost. The pattern of clearance of IgG sensitized cells by the liver was typical of that noted for IgG (Fig. 7). This indicates that the patterns of clearance of IgG- and IgM-coated cells are defined by the class of antibody rather than by the organ responsible for the clearance.

IgG sensitized cells cleared from the circulation in splenectomized guinea pigs were found in the liver (Table III); there was never significant uptake by the lungs

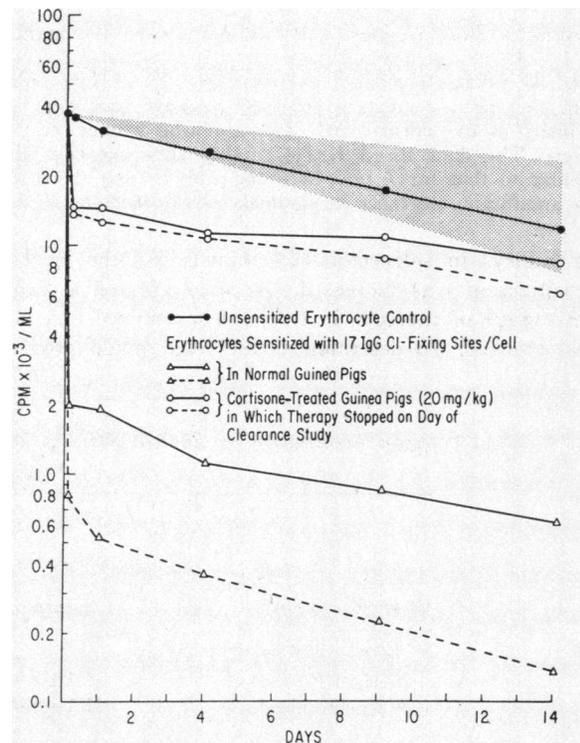


FIGURE 5 Survival of ^{51}Cr -labeled guinea pig erythrocytes sensitized with 17 IgG C1-fixing sites per cell: comparison between two normal and two corticosteroid-treated guinea pigs. Cortisone therapy was discontinued on day one in this and many similar experiments. Following the initial clearance period, the survival of sensitized erythrocytes in these guinea pigs parallels that observed for sensitized and unsensitized erythrocytes in normals.

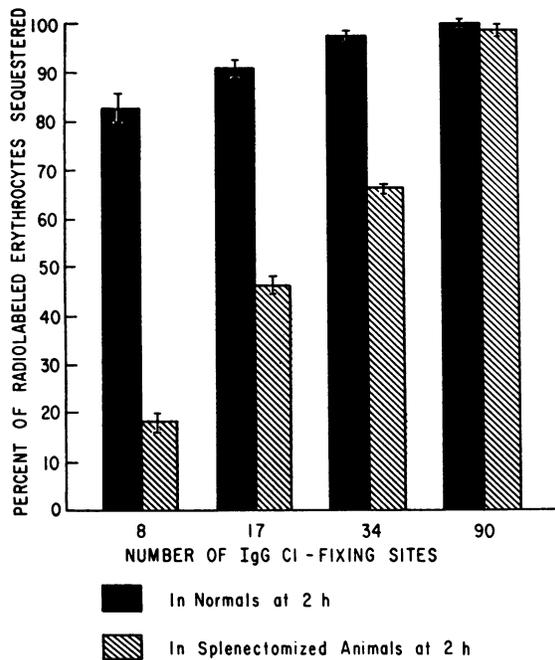


FIGURE 6 Dose response relationship between the number of IgG C1-fixing sites and the extent of clearance in splenectomized animals compared with normals. These data represent the mean \pm standard error of at least four and two animals in the normal and splenectomized groups, respectively. The data for 3 IgG C1-fixing sites per cell were similar to that for 7 IgG C1-fixing sites in that there was no significant clearance by animals postsplenectomy.

or kidneys in splenectomized animals. As the level of sensitization was increased, the liver cleared a larger percentage of the cells in both splenectomized and normal animals. To determine if the liver would progres-

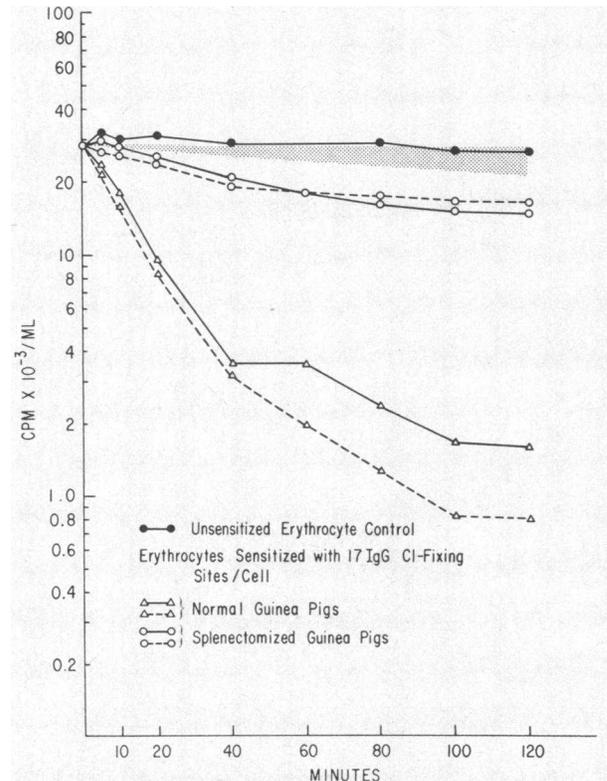


FIGURE 7 Survival of ⁵¹Cr-labeled guinea pig erythrocytes sensitized with 17 IgG C1-fixing sites per cell: comparison between two normal and two splenectomized guinea pigs.

sively increase its sequestering ability following splenectomy, clearance studies using erythrocytes sensitized with 17 IgG C1-fixing sites were performed at 1, 2, 4, and 8 wk postsplenectomy. There was no evidence that

TABLE III
Organ Localization of Radiolabeled Sensitized Erythrocytes in Splenectomized Compared with Normal Guinea Pigs*

	Circulation	Organ			
		Liver	Spleen	Kidneys	Lungs
Unsensitized control (15)†	8.0 \pm 4.6	8.0 \pm 4.1	3.1 \pm 1.3	1.1 \pm 0.8	3.4 \pm 1.4
17 IgG C1-fixing sites					
Normal (6)	6.1 \pm 1.9	24.1 \pm 1.8	68.3 \pm 2.6	1.0 \pm 0.6	1.8 \pm 0.3
Splenectomized (2)	57.1 \pm 5.6§	37.4 \pm 4.9§		2.9 \pm 1.4	3.6 \pm 0.9
34 IgG C1-fixing sites					
Normal (4)	0.3 \pm 0.1	42.3 \pm 3.9	56.8 \pm 4.1	0.9 \pm 0.5	0.3 \pm 0.1
Splenectomized (2)	33.5 \pm 3.5§	58.4 \pm 0.4§		2.4 \pm 1.1	4.1 \pm 0.7

* Mean percent \pm standard error 2 h after injection. No attempt has been made to correct for radioactivity of blood within organs.

† Number of animals in each group.

§ $P < 0.05$ when compared with normal animals at the same level of sensitization.

the liver acquired increased ability to clear IgG-coated cells.

Effect of cortisone acetate on the hematocrit, body weight, and spleen and liver weights. Hematocrits and weights were obtained on saline- and cortisone-treated guinea pigs before and after each clearance study. At no time was the hematocrit outside of the normal range (38 ± 4). Groups of animals receiving high dose cortisone acetate, followed for 14 days, had a percentage weight gain which was not significantly different from untreated controls ($P > 0.05$). There was a slight but not significant reduction in splenic weight ($P > 0.05$). The livers in the high dose and low dose cortisone group were 1.7 and 1.3 times as large, respectively, as those of the untreated group. The increase in liver weight has previously been shown to be secondary to glycogen accumulation (12).

Effect of in vitro preincubation of IgG-sensitized erythrocytes in 100 $\mu\text{g}/\text{ml}$ of hydrocortisone sodium succinate on clearance. Incubation of erythrocytes sensitized with 3 or 17 IgG C1-fixing sites with hydrocortisone did not alter the clearance pattern as compared with controls. In addition, clearance patterns did not differ from those of appropriate controls when erythrocytes were incubated in cortisone buffer for 30 min before and during sensitization.

DISCUSSION

These experiments were performed in order to define in molecular terms the effect of corticosteroid therapy and of splenectomy on the clearance of IgG- and IgM-coated erythrocytes. Corticosteroid therapy increased the survival of IgG- and IgM-sensitized erythrocytes by decreasing sequestration in the reticuloendothelial system (RES). The RES of cortisone-treated animals appeared to have a lowered sensitivity to erythrocytes coated with antibody and complement since sensitized erythrocytes were removed at rates consistent with fewer C1-fixing sites per cell. As the number of IgG C1-fixing sites was increased, the difference in erythrocyte survival between cortisone-treated and control animals was less marked. However, with IgM, increasing the level of sensitization did not markedly diminish the effectiveness of corticosteroids. The general pattern and kinetics of clearance were not modified by corticosteroids. Sites of sequestration remained the same in cortisone-treated animals and controls.

Several reports in the clinical and experimental literature bear on these findings. Kaplan and Jandl (13) examined the clearance of antibody-coated cells in rats utilizing antisera prepared in rabbits and exposing the sensitized cells to fresh rat serum as 40% suspension prior to injection. They found that cortisone acetate therapy decreased sequestration of cells at all levels of

sensitization studied. This effect was first noted after 3 days of treatment and was most marked after 5-6 days. Their data indicated that the increased survival of erythrocytes in cortisone-treated rats was secondary to decreased hepatic uptake. They found no decrease in splenic sequestration and postulated that alterations in hepatic blood flow induced by cortisone might explain their results. Our findings in the guinea pig are similar as to time of onset of the steroid effect but differ in several important aspects. In the studies reported here hepatic sequestration of IgM-coated cells and also splenic sequestration of IgG-coated cells were affected by cortisone acetate. Moreover, in these studies, the ability of cortisone therapy to increase the survival of IgG-sensitized cells was inversely related to the level of sensitization. Some of these differences probably relate to the fact that Kaplan and Jandl (13) utilized unfractionated antisera which may contain both IgG and IgM antibody. The sites of sequestration in the case of IgG-coated cells are dependent on the number of antibody molecules coating the cell. At lower numbers of antibody molecules, splenic sequestration predominates while at higher numbers hepatic sequestration is most evident (8, 14, 15). Another important difference is that the guinea pig, unlike the rat, resembles man in being able to maintain body weight, spleen size, and albumin and globulin levels while being treated with as much as 50 mg/kg of cortisone acetate (12). The rat loses weight, develops splenic atrophy and may suffer a significant mortality. Our data confirm the hypothesis that cortisone therapy decreases reticuloendothelial sequestration of sensitized cells. It is obvious that redistribution of hepatic blood flow cannot explain the findings reported here. Rather the most likely explanation for the cortisone effect is a decreased sensitivity of the RES for erythrocytes coated with antibody and complement; cells with 17 IgG C1-fixing sites per cell are cleared as if they had 3 sites per cell. The mechanism for these cortisone effects is unknown. However, if cortisone therapy decreased the rate of synthesis of macrophage membrane receptors, this could account for the experimental observation of a decreased avidity of the RES for sensitized cells. Interestingly, in studies not detailed here, the clearance of cells heavily sensitized with IgG antibody was decreased in C4-deficient guinea pigs. Since in these animals clearance is not complement dependent (9), this finding suggests decreased recognition of IgG sites as well as complement sites by the RES in cortisone-treated animals.

Mollison (16) injected Rh-sensitized cells into patients before and after 5-6 days of cortisone therapy (100 mg/day) and the rate of destruction was determined. In only two out of five cases did this therapy appear to slow the rate of clearance of antibody-coated cells. Part of the reason for the equivocal results re-

lates to the short duration and low dose of cortisone employed. However, as Mollison suggested, the most important reason for these results was probably that the erythrocytes were treated with a relatively large quantity of antibody. We have shown that the efficacy of corticosteroids in preventing clearance of IgG-sensitized cells is inversely related to the number of IgG antibody molecules coating the cell. Clearance of Rh antibody-sensitized cells is reported to be noncomplement dependent and this may represent another difference between the study model (16) and our own.

Rosse (3) recently reported a group of patients with autoimmune hemolytic anemia in whom he measured the amount of cell-bound antibody before and during corticosteroid therapy. In one patient a remission was induced by corticosteroid therapy and maintained despite the fact that the amount of cell-bound antibody remained the same. Thus, in this patient corticosteroids appeared to induce a remission by preventing sequestration. Most of these patients, however, had a rapid decrease in cell-bound antibody during therapy while concomitantly showing an increase in free circulating antibody. In the studies reported herein we attempted to show a direct effect of corticosteroid therapy on the binding of rabbit antibodies to guinea pig erythrocytes. We were unable to demonstrate an altered clearance pattern following *in vitro* incubation of sensitized cells with cortisone; however, these antigen-antibody systems may not be comparable.

The effectiveness of corticosteroids in preventing hepatic sequestration of IgM-sensitized cells is somewhat surprising since in most patients this therapy has been disappointing (1, 2, 17). However, our studies were in a nonlytic system at higher levels of corticosteroid therapy than are generally used in clinical medicine (17). Our data suggest that rather high doses of corticosteroids (100-300 mg prednisone/day) might be considered in seriously ill patients with cold agglutinin mediated, Coombs positive hemolytic anemia.

These experiments also examined the role of splenectomy in cell clearance. No effect at all was noted on the clearance of IgM-sensitized cells. This result might be expected since these cells are cleared by the liver (1, 8, 17, 18). Splenectomy is known not to be efficacious in treatment of patients with IgM-coated erythrocytes as noted in the cold hemagglutinin syndrome (1, 17).

Splenectomy led to an increased survival of IgG-sensitized erythrocytes at all levels below 90 C1-fixing sites per cell. As the number of IgG C1-fixing sites was increased from 3 to 90 per cell, the liver removed a progressively higher percentage of the cells in normals and splenectomized animals. However, clearance of IgG sensitized cells by the liver in splenectomized animals was less efficient than clearance by the spleen in normals

at similar levels of sensitization. A sixfold increase in IgG C1-fixing sites was necessary to obtain similar rates of clearance in splenectomized and control animals. Splenectomy was more effective than corticosteroids in preventing IgG-coated cells from destruction by the RES at equivalent levels of sensitization. Clearance studies performed from 1 to 8 weeks post-splenectomy did not reveal any evidence that the liver acquired greater capacity to clear IgG-sensitized cells over the course of time. In this model the animals did not have an ongoing hemolytic process to stress the RES which might be necessary for the liver to increase its sequestering ability (19, 20).

The data relating to the clearance of sensitized erythrocytes in splenectomized hosts are consistent with the limited number of available experimental and clinical studies. It has been shown in studies of splenectomized patients that IgG-sensitized cells are cleared by the liver and that higher levels of sensitization are required in splenectomized patients for equivalent degrees of clearance (16, 18). It is known from clinical studies that patients relapsing after splenectomy for IgG mediated hemolytic anemia sequester erythrocytes in the liver. In a recent study of the numbers of antibody molecules coating erythrocytes in autoimmune hemolytic anemia, the highest level of cell bound antibody was found in previously splenectomized patients (3). Thus, these clinical studies are consistent with the data herein, demonstrating the liver to be much less efficient at removing IgG-sensitized erythrocytes.

Prior to the use of corticosteroids, clinical experience suggested that splenectomy was quite effective at inducing a remission in patients with AIHA but relapse was common (1, 2). The initial response of patients to splenectomy appears easily explained in that the primary site of sequestration is removed. If antibody synthesis continues, it is possible that circulating cells in these patients may gradually acquire higher levels of sensitization. A point may come when the level of sensitization is sufficient to lead to liver sequestration and then relapse may occur.

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