

Effects of Corticosteroids on Immunity in Man I. DECREASED SERUM IgG CONCENTRATION CAUSED BY 3 OR 5 DAYS OF HIGH DOSES OF METHYLPREDNISOLONE

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J Clin Invest. 1973;**52**(10):2629-2640. <https://doi.org/10.1172/JCI107455>.

Research Article

To study the effects of methylprednisolone on immune mechanisms in the absence of other immunosuppressive agents or immunologically mediated diseases, we gave 17 normal adult male volunteers 96 mg of methylprednisolone daily for 3-5 days and compared results with 12 untreated controls who were studied simultaneously, 86% of treated volunteers had significant decreases in the concentrations of serum IgG. 2-4 wk after methylprednisolone, the treated volunteers had a mean decrease in IgG of 22% compared with a decrease of only 1% in untreated controls. Likewise, significant decreases in IgA concentration occurred in 43% of treated volunteers, whereas significant decreases in IgM occurred in only 14%. The lowest immunoglobulin levels occurred during the 2nd wk after a 3 day course of methylprednisolone and during the 3rd wk after a 5 day course of drug. Slightly decreased plasma concentration of [125 I]IgG was seen in six of seven volunteers who received a 5 day course but in only one of four who received a 3 day course of drug. However, an increase in the rate of plasma clearance of IgG occurred only during the treatment period itself. During the period when the serum concentration of IgG was falling, the specific activity of IgG in the serum was relatively higher in treated men than in controls indicating decreased entry of newly synthesized IgG into [...]

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Effects of Corticosteroids on Immunity in Man

I. DECREASED SERUM IgG CONCENTRATION CAUSED BY 3 OR 5 DAYS OF HIGH DOSES OF METHYLPREDNISOLONE

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ABSTRACT To study the effects of methylprednisolone on immune mechanisms in the absence of other immunosuppressive agents or immunologically mediated diseases, we gave 17 normal adult male volunteers 96 mg of methylprednisolone daily for 3–5 days and compared results with 12 untreated controls who were studied simultaneously. 86% of treated volunteers had significant decreases in the concentrations of serum IgG. 2–4 wk after methylprednisolone, the treated volunteers had a mean decrease in IgG of 22% compared with a decrease of only 1% in untreated controls. Likewise, significant decreases in IgA concentration occurred in 43% of treated volunteers, whereas significant decreases in IgM occurred in only 14%. The lowest immunoglobulin levels occurred during the 2nd wk after a 3 day course of methylprednisolone and during the 3rd wk after a 5 day course of drug. Slightly decreased plasma concentration of [125 I]IgG was seen in six of seven volunteers who received a 5 day course but in only one of four who received a 3 day course of drug. However, an increase in the rate of plasma clearance of IgG occurred only during the treatment period itself. During the period when the serum concentration of IgG was falling, the specific activity of IgG in the serum was relatively higher in treated men than in controls indicating decreased entry of newly synthesized IgG into the circulation. These findings suggest that a short course of methylprednisolone treatment causes a pronounced and sustained decrease in serum IgG due to increased catabolism during drug administration and to decreased synthesis during and for a variable time after drug administration.

Received for publication 26 June 1972 and in revised form 14 May 1973.

INTRODUCTION

Although corticosteroids are known to suppress both cell-mediated and humoral immune responses in experimental animals (1–6), their effects on immunity in man are only poorly understood (3, 4). At the time when the effects in man were last studied systematically 20 yr ago, investigators employed relatively low doses of ACTH and cortisone acetate compared with present-day usage (3). In general, they were unable to detect significant effects on human immunity (3). Comparable studies have not been done using the newer synthetic corticosteroids. Moreover, systematic studies of the effects on immune responsiveness of different corticosteroids, dose, route of administration, and timing of treatment in relation to administration of antigen have not been done.

The discrete effects of corticosteroids on immunity are often difficult to study in individual patients. Patients who are treated with high doses of potent corticosteroids often receive other immunosuppressive agents concurrently. These same patients may in addition have preexisting immunologic abnormalities that tend to mask the specific effects of corticosteroids. Patients with systemic lupus erythematosus, for example, may exhibit hyperresponsiveness to nuclear and membrane antigens (7) and hyporesponsiveness to other antigens (8), whereas both cell-mediated and humoral responses are usually suppressed in recipients of renal allografts (9) and in many patients with malignancies (10). Finally, corticosteroids have many biologic actions and the manner in which they influence cell destruction, phagocytosis, inflammation, cellular metabolism, and protein synthesis may also alter immune responsiveness in ways that are not yet well understood (3–6).

In order to better understand how corticosteroids affect immunological responses in man, we gave normal volunteers 96 mg of methylprednisolone daily in divided doses for up to 5 days. We measured the effect of this treatment on various aspects of humoral and cell-mediated immunity. This report presents results of experiments which indicate that 3 or 5 days of methylprednisolone caused a decrease in serum IgG in 12 of 14 volunteers. The maximum effect occurred 2–3 wk after drug treatment. In four cases tested after 3 mo, IgG concentrations were still significantly decreased.

METHODS

Volunteers. Subjects were 29 normal adult males admitted for 4–6 wk periods to the General Clinical Research Center at The Methodist Hospital as participants in a volunteer program established in cooperation with the Texas Department of Corrections. To be included in the study, a candidate must have been within normal limits with respect to history, physical examination, roentgenographic examination of the chest and sinuses, electrocardiogram, white blood-cell count and differential, hemoglobin and hematocrit, reticulocyte count, platelet count, serum sodium, potassium, chloride, carbon dioxide, calcium, amylase, bilirubin, transaminases, lactic dehydrogenase, prothrombin time, alkaline phosphatase, protein electrophoresis, immunoelectrophoresis, and creatinine, blood urea nitrogen, fasting blood sugar, glucose tolerance after 100 g of glucose with blood, and urine glucose determinations at $\frac{1}{2}$, 1, 2, and 3 h, serologic test for syphilis, urinalysis, bacterial culture of throat and urine, and examination of stool for ova and parasites and for occult blood. In addition, the subjects must have been negative for Australia antigen and negative to testing with the intermediate strength of tuberculin purified protein derivative (PPD). Many of the above studies were repeated during and periodically after steroid administration; essentially no changes occurred except in white blood-cell counts and differentials, glucose tolerance tests and protein electrophoretic analyses, the latter of which will be described elsewhere (11).

Experimental procedure. All experiments included simultaneously conducted studies in treatment and control groups. Volunteers were randomly assigned to the study groups except that the eight volunteers who had positive PPD skin test reactions were assigned to control groups. Otherwise, the control and test groups were comparable in skin-test responsiveness to candida, dermatophytin, mumps, and histoplasmin. Results of three separate experiments are reported here: In experiment A, four volunteers served as controls and eight received methylprednisolone, four for 3 days and four for 5 days. In experiment B, six volunteers served as controls and six received drug for 5 days. In experiment C, two were controls and three received treatment for 3 days. Except for treatment with methylprednisolone, treatment and control groups were identical in all respects, that is, in amount and timing of blood samples drawn, immunization, skin testing, etc. All subjects were under close observation by the nursing staff and were examined frequently by physicians throughout the study. No one was allowed to leave the clinical research center during a study.

Drug administration. 16 mg of methylprednisolone (Medrol, The Upjohn Co., Kalamazoo, Mich.) was given orally every 4 h for either 3 or 5 days. No serious untoward

reactions occurred. An occasional volunteer complained of anorexia which occurred only during the treatment period. All volunteers, including controls, were given 30 ml of an antacid orally (Gelusil, Warner-Chilcott Laboratories, Morris Plains, N. J.) containing aluminum hydroxide and magnesium trisilicate three times daily throughout the period of administration of methylprednisolone.

Immunoglobulin and protein assays. The concentrations of IgG, IgA, and IgM were measured by single radial diffusion in agar using commercially prepared plates (Melay Laboratories Inc., Springfield, Va.). Up to 20 serial specimens obtained during a 5–6 wk period from individual volunteers were analyzed in block titration along with appropriate standards on the same agar plate. The standard deviations of 24 replicate immunoglobulin determinations run on the same plates were ± 0.4 mg/ml for IgG, ± 0.04 mg/ml for IgA, and ± 0.03 mg/ml for IgM, respectively. In experiment B, we also obtained follow-up bloods after 3 mo from four of the six methylprednisolone-treated volunteers who were still available for study. The immunoglobulin assays on these late sera were carried out in block along with pre- and posttreatment sera that had been kept at -70°C .

Radioisotopic procedures. 29 tracer experiments were done, 12 in controls, and 17 in treated volunteers. The tracer dose was given to 3 volunteers before drug treatment, 11 volunteers at the start of treatment, and three volunteers after treatment had been completed. All isotopic injections in each experiment were given within a 1 h period on the same day.

IgG was extracted from freshly drawn normal human serum by electroconvection (12) and labeled with ^{125}I by the iodine monochloride method (13). Three separate, radioiodinated normal human IgG preparations were used, one each for experiments A, B, and C, respectively. The preparation used in experiment A contained an average of 2.2×10^{-8} atoms of iodine per IgG molecule, in experiment B, 2.9×10^{-8} atoms, and experiment C, 2.3×10^{-8} atoms. After labeling, 20 mg of human serum albumin per ml was added and the mixture was passed through a sterile $0.22 \mu\text{m}$ Millipore filter (Millipore Corp., Bedford, Mass.) and determined to be pyrogen and bacteria free. 48 h before administration of the ^{125}I -IgG, volunteers in both control and treatment groups were begun on Lugol's solution, 0.5 ml three times daily, to block thyroid uptake of free radioiodine. Lugol's solution was continued throughout the hospitalization. Approximately 50 μCi of the labeled IgG was injected intravenously in one arm, and exactly 10 min later, a blood sample was taken from the other arm and used as the zero-time sample. The plasma disappearance curves were determined as described previously (14, 15).

To compare IgG degradation during control and drug treatment periods, we measured both the plasma clearance of ^{125}I -IgG and the urinary loss of ^{125}I . The ^{125}I excreted in the urine in 24 h (cpm per ml urine \times ml urine per 24 h) was taken to represent ^{125}I released by metabolic degradation of ^{125}I -IgG. This value was divided by the counts per minute of ^{125}I -IgG in the plasma at the midpoint of the 24 h urine collection period to obtain a value that represented the "metabolic clearance" of ^{125}I -IgG (16). The "metabolic clearance" of ^{125}I -IgG was then normalized for body weight and expressed as milliliters of plasma cleared per 24 h per kilogram body weight. This method was used because its validity is independent of the steady state (16).

The decreases in IgG concentrations were analyzed in terms of expected and observed results (see Discussion and Fig. 12). To make the calculations for expected results,

TABLE I
Effect of Methylprednisolone on Serum IgG, IgA, and IgM

Volunteer code	Methylprednisolone treatment	IgG concentration					IgA concentration					IgM concentration				
		Posttreatment (days 14-28 only)					Posttreatment (days 14-28 only)					Posttreatment (days 14-28 only)				
		Pre-treatment	Mean value*	Sera tested	Net change	Percent change	Pre-treatment	Mean value*	Sera tested	Net change	Percent change	Pre-treatment	Mean value*	Sera tested	Net change	Percent change
	days	mg/ml	mg/ml	no.	mg/ml	%	mg/ml	mg/ml	no.	mg/ml	%	mg/ml	mg/ml	no.	mg/ml	%
V	5	16.0	10.8	11	-5.2	-32.5	3.20	2.48	4	-0.72	-22.5	1.30	1.16	5	-0.14	-10.8
J	5	13.0	11.3	6	-1.7	-13.1	3.50	3.20	2	-0.30	-8.6	1.30	1.30	2	0.0	0.0
F	3	13.0	9.3	6	-3.7	-28.5	1.40	0.96	2	-0.44	-31.4	0.79	0.79	2	0.0	0.0
P	5	12.5	7.4	6	-5.1	-40.8	1.25	0.83	5	-0.42	-33.6	2.16	1.58	5	-0.58	-26.9
E	3	12.0	10.3	6	-1.7	-14.2	1.35	1.35	2	0.0	0.0	1.80	1.48	2	-0.32	-17.8
K	5	12.0	9.2	6	-2.8	-23.3	2.00	1.80	2	-0.20	-10.0	1.30	1.17	2	-0.13	-10.0
X	5	11.3	8.9	12	-2.4	-21.2	1.05	0.92	6	-0.13	-12.4	0.90	1.04	6	+0.14	+15.6
I	5	11.2	11.2	6	0.0	0.0	1.80	1.80	2	0.0	0.0	2.30	2.30	2	0.0	0.0
L	5	11.2	6.5	6	-4.7	-42.0	1.70	1.60	2	-0.10	-5.9	1.80	1.25	2	-0.55	-30.6
W	5	10.5	7.8	10	-2.7	-25.7	1.10	0.88	6	-0.22	-20.0	1.40	1.25	6	-0.15	-10.7
H	3	9.8	8.7	6	-1.1	-11.2	1.35	1.17	2	-0.18	-13.3	0.79	0.89	2	+0.10	+12.7
Q	5	9.3	6.2	6	-3.1	-33.3	1.00	0.67	5	-0.33	-33.0	1.50	1.41	4	-0.09	-6.0
G	3	9.0	9.3	6	+0.3	+3.3	1.35	1.13	2	-0.22	-16.0	2.25	2.25	2	0.0	0.0
R	5	6.1	4.5	6	-1.6	-26.2	0.65	0.51	5	-0.14	-21.5	1.70	1.59	5	-0.11	-6.5
Averages		11.2	8.7		-2.5	-22.1	1.62	1.38		-0.24	-16.3	1.52	1.39		-0.13	-6.5
M	None	18.3	17.9	5	-0.4	-2.2	0.95	0.84	5	-0.11	-11.6	2.40	2.25	4	-0.15	-6.3
T	None	16.0	14.8	10	-1.2	-7.5	1.70	1.67	5	-0.03	-1.8	1.40	1.38	5	-0.02	-1.4
A	None	15.0	15.0	2	0.0	0.0	1.20	1.20	2	0.0	0.0	1.20	1.20	2	0.0	0.0
B	None	14.0	14.0	2	0.0	0.0	1.60	1.33	2	-0.27	-16.9	1.80	1.70	2	-0.10	-5.6
C	None	11.3	11.5	6	+0.2	+1.8	ND†	ND†				1.40	1.25	2	-0.15	-10.7
D	None	10.5	10.5	2	0.0	0.0	0.76	0.72	2	-0.04	-5.3	1.40	1.50	2	+0.10	+7.1
S	None	10.5	10.6	11	+0.1	+1.0	1.20	1.13	6	-0.07	-5.8	0.75	0.93	6	+0.18	+24.0
N	None	10.4	10.0	5	-0.4	-3.8	1.75	1.94	4	+0.19	+10.9	1.95	2.00	4	+0.05	+2.6
U	None	9.6	9.5	10	-0.1	-1.0	1.50	1.28	6	-0.22	-14.7	1.00	0.73	6	-0.27	-27.0
O	None	8.7	8.6	6	-0.1	-1.1	1.35	1.25	4	-0.10	-7.4	1.60	1.55	4	-0.05	-3.1
Averages		12.4	12.2		-0.2	-1.3	1.33	1.26		-0.07	-5.8	1.49	1.45		-0.04	-2.0

* The posttreatment mean value is the average of all sera tested between days 14 and 28, the exact number indicated in the adjacent column.

† ND, not done.

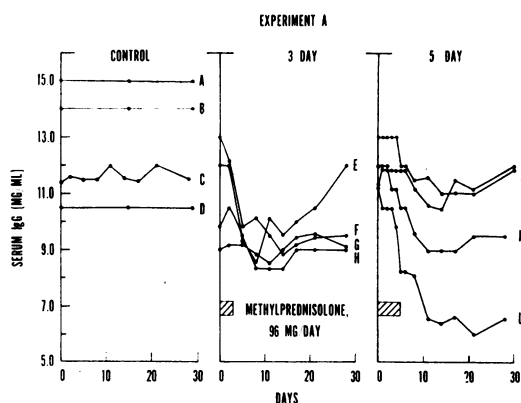


FIGURE 1 Dose effect of methylprednisolone treatment on IgG concentration. Serial IgG determinations demonstrate the lag phase before IgG concentrations begin to decline rapidly and the delayed onset of recovery in the 5 day treatment group. Code letters represent individual volunteers.

the following assumptions and conditions were made: (a) body weight = 70 kg, (b) total IgG pool = 1,060 mg/kg (17), (c) total i.v. pool of IgG = 510 mg/kg (17), (d) fractional catabolic rate = 6.3% of i.v. pool/day (17), (e) IgG synthetic rate = 32 mg/kg/day (17), (f) normal serum IgG = 12 mg/ml, (g) catabolism occurs from the circulating pool, (h) rapid equilibrium occurs between intra- and extra-vascular pools, (i) no compensatory mechanisms take place to alter normal synthetic or catabolic rates, and (j) daily IgG loss from blood drawing = 6 mg IgG/kg/day. The following conditions related to methylprednisolone treatment were established: (a) during the treatment period, the fractional catabolic rate of IgG increase by 50%, (b) there is a lag phase of 2 days before inhibition of synthesis is manifested, (c) synthesis of IgG is inhibited according to three different conditions indicated in Fig. 12.

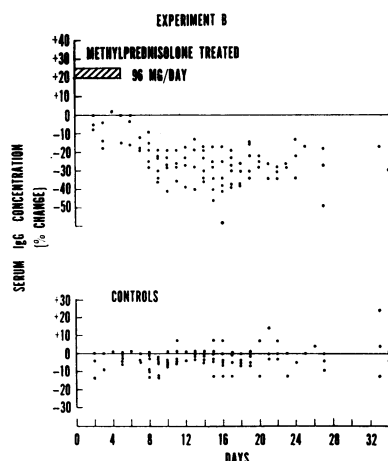


FIGURE 2 Effect of methylprednisolone on serum IgG concentrations in six treated and six control volunteers. Points represent results of IgG assays on individual serum samples.

RESULTS

Changes in serum concentrations of IgG, IgA, and IgM

Serial immunoglobulin concentrations were measured in the 10 volunteers who received a 5 day course and in the 4 who received a 3 day course of methylprednisolone in experiments A and B. Serial immunoglobulin levels were not measured in experiment C. The data are presented in three ways: (a) Table I summarizes the net changes in IgG, IgA, and IgM concentrations in all control and treated volunteers in experiments A and B. (b) Fig. 1 shows the serial IgG concentrations of individual volunteers in experiment A, and (c) Figs. 2-4, respectively, show serial IgG, IgA, and IgM concentrations of grouped data from experiment B.

Serum IgG. Control volunteers showed minimum fluctuations in IgG concentrations. The average net change in IgG concentration over a period of 4 wk was minus 1.3% and the maximum individual decrease was 7.5%. On the other hand, 12 or 86% of the 14 treated volunteers had net decreases in serum IgG that exceeded the maximum decrease observed in the control group. The average net change in the 14 treated volunteers was minus 22.1%; there was considerable individual variation and four volunteers had net decreases exceeding 30.0%. The magnitude of the decreases was not related to the pretreatment concentrations as can be seen by examining the ranked data in Table I. Two volunteers failed to show appreciable net changes (volunteers G and I). On the other hand, when their individual serial values are examined in Fig. 1, these two men had IgG fluctuations

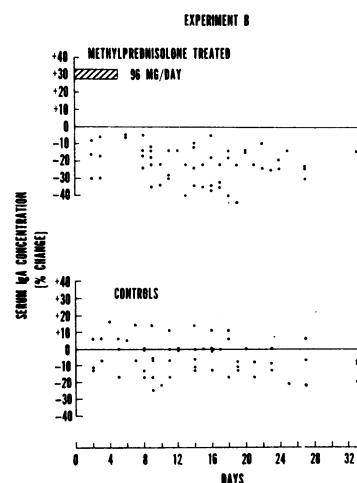


FIGURE 3 Effect of methylprednisolone on serum IgA concentrations in six treated and six control volunteers. Points represent results of IgA assays on individual serum samples. Notes decrease in IgA during drug treatment in several volunteers.

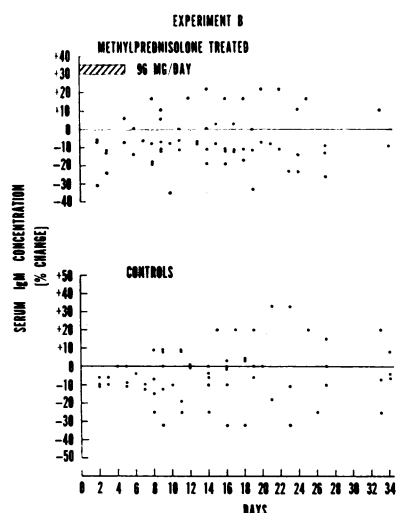


FIGURE 4 Effect of methylprednisolone on serum IgM concentrations in six treated and 6 control volunteers. Points represent results of IgM assays on individual serum samples. Note decrease in IgM during drug treatment in two volunteers and lack of difference between drug treated and control groups after treatment.

which slightly exceeded those seen in the controls, possibly suggesting a minimal, but transitory effect.

Serum IgA. IgA concentrations also decreased after treatment with methylprednisolone. As seen in Fig. 3, significant decreases occurred early in the drug treatment period in several volunteers. Serum IgA concentrations fluctuated more widely in both treated volunteers and controls than had been observed with IgG. Of the 14 treated volunteers, only 6 (43%) had net decreases in the 4 wk observation period exceeding the maximum variations seen in the controls.

Serum IgM. As Table I indicates, the changes within the control group were even more striking than seen with IgA, ranging from a net increase of 24.0% to a net decrease of 27.0%. Looking at the grouped data from experiment B shown in Fig. 4, there are no readily apparent differences between the treatment and control groups, except possibly for the very early decreases seen in several volunteers during the drug treatment period. No group trends emerged as was seen with IgG and IgA. When the data were examined on an individual basis, however, it seems likely that the changes in IgM observed in two of the volunteers (L and P) were due to drug treatment since these two volunteers also had the greatest decreases in IgG (Tables I and II).

Temporal relationship between administration of methylprednisolone and initial fall in immunoglobulin concentrations. IgG levels remained constant for 2–6 days after starting methylprednisolone and then began to decrease, in some cases, quite abruptly (Fig. 1). In volunteers who received a 3 day course of methylpred-

nisolone, IgG concentrations leveled out during the 2nd wk; thereafter there was an upward trend. In contrast, after a 5 day course of drug, depression of immunoglobulin levels was more prolonged, and onset of recovery was not apparent in some cases until the 4th wk. The volunteers with the largest percent decrease in IgG concentration also had the slowest rate of return to normal circulating concentrations of IgG ($r = 0.86$, $P < 0.001$, [18]).

As seen in Figs. 3 and 4, two volunteers in experiment B developed highly significant decreases in IgA and IgM within 2 days of starting methylprednisolone. One of the eight treated volunteers in experiment A demonstrated a 24% decrease in IgM by the 4th day of treatment. Thus, abrupt early decreases in IgA and IgM were the exception rather than the rule. However, it is important to note that these early changes in IgM or IgA were not associated with significant changes in IgG. As shown in Fig. 5, volunteer P developed a 31% decrease in IgM and a 16% decrease in IgA by the

TABLE II
Comparison of Changes in IgG Concentrations to Changes in IgA and IgM Concentrations after Methylprednisolone*

Volunteer code	Percent change from pretreatment value†		
	IgG	IgA	IgM
Methylprednisolone treated			
L	-42.0	-5.9	-30.6
P	-40.8	-33.6	-26.9
Q	-33.3	-33.0	-6.0
V	-32.5	-22.5	-10.8
F	-28.5	-31.4	0
R	-26.2	-21.5	-6.5
W	-25.7	-20.0	-10.7
K	-23.3	-10.0	-10.0
X	-21.2	-12.4	+15.6
E	-14.2	0	-17.8
J	-13.1	-8.6	0
H	-11.2	-13.3	+12.7
I	0	0	0
G	+3.3	-16.0	0
Controls			
T	-7.5	-1.8	-1.4
N	-3.8	+10.9	+2.6
M	-2.2	-11.6	-6.3
O	-1.1	-7.4	-3.1
U	-1.0	-14.7	-27.0
A	0	0	0
B	0	-16.9	-5.6
D	0	-5.3	+7.1
S	+1.0	-5.8	+24.0
C	+1.8	ND‡	-10.7

* Posttreatment values are based on mean value of all sera tested 14–28 days after methylprednisolone (see Table I).

† Statistical testing by the Spearman rank-order correlation test (18). (a) Methylprednisolone-treated volunteers: IgG vs. IgA, $r = 0.54$, $P < 0.05$; IgG vs. IgM, $r = 0.67$, $P < 0.01$.

(b) Controls: IgG vs. IgA, $r = 0.1$; $P > 0.1$; IgG vs. IgM, $r = 0.07$, $P > 0.1$.

‡ ND = Not done.

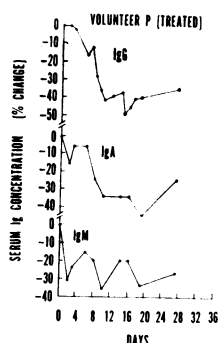


FIGURE 5 IgG, IgA, and IgM concentrations in sera of volunteer P treated for 5 days with methylprednisolone. On day 2 of treatment, IgA and IgM concentrations decreased significantly, whereas there was no change in IgG concentration.

2nd day of treatment. In contrast, there was no change in the concentration of IgG in the serum sample from that day.

Duration of drug-induced effect. On the 28th day after methylprednisolone, the IgG concentrations remained significantly decreased in 8 of the 10 volunteers

who received 5 days and in only 1 of the 4 who received 3 days of drug treatment indicating the dose effect mentioned above. 3 mo after administration of methylprednisolone, IgG concentrations had returned further toward normal in the four volunteers studied, but were still between 8 and 30% below base-line values. A persistent decrease in IgM was seen in only one of the four volunteers after 3 mo whereas IgA concentrations had returned to normal in all four men.

Relationship of changes in IgG to those of IgA and IgM. Table II shows that there was a tendency for volunteers who developed significant decreases in IgG to also develop decreases in IgA and to IgM, regardless of whether the latter decreases were considered significant when compared with controls. In contrast, changes in the three immunoglobulin classes within the control group were independent of each other.

Weight changes. Measurement of body weights in volunteers in experiment A indicated that 2 days after a 5 day course of methylprednisolone, mean body weight had increased by 4.1% (Fig. 6l). Continuing over the next 5-7 day period, the volunteers then lost about one-half of the 4.1% gained. Similar trends but of lesser magnitude were seen in the volunteers treated for 3 days (Fig. 6k). Note that changes also occurred in volunteers G and I, the two volunteers who failed to develop significant changes in IgG concentrations. Control volunteers showed a slight but steady increase in weight reaching a mean of 4% by the 4th wk of observation. At the time corresponding to the day of peak weight gain in treated volunteers, the mean increase in weight in the controls was only 0.9% (Fig. 6j).

IgG metabolism studied after intravenous injection of [125 I] normal human IgG

Plasma survival of [125 I]IgG. 11 volunteers were given isotopically labeled IgG at the beginning of drug treatment, 4 at the start of a 3 day course and 4 at the start of a 5 day course of drug in experiment A, and 3 at the beginning of a 5 day course in experiment B. Slightly decreased plasma concentration of injected [125 I]-IgG was seen in six of the seven volunteers who received a 5 day course of methylprednisolone (Figs. 6f and 7a) but in only one of the four volunteers who received 3 days of drug (Fig. 6e). With the exception of volunteer Q (Fig. 7a) whose plasma clearance of [125 I]IgG was more rapid from the outset, the decreased plasma concentration became obvious only during the week after treatment.

Three additional volunteers were given a tracer dose of IgG 10 days before a 3 day course of methylprednisolone. The drug treatment did not significantly alter the plasma survival of the [125 I]IgG (Fig. 8a). Finally, when methylprednisolone was given for 5 days to three

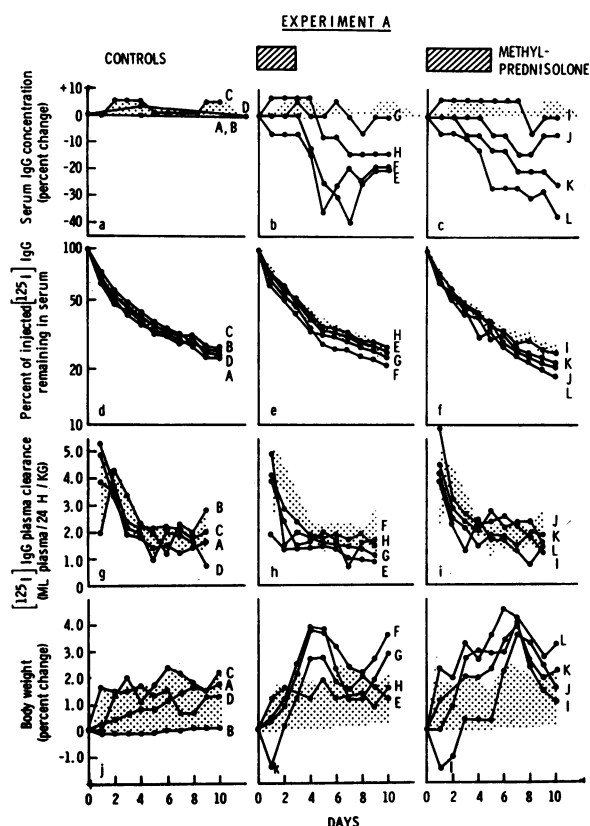


FIGURE 6 Plasma survival and clearance of intravenously injected [125 I]IgG compared with changes in serum IgG concentrations and changes in body weight.

other volunteers beginning 8 days before the tracer dose of IgG, the plasma survival of the IgG was similar to that in the controls (Fig. 7b).

Metabolic clearance of [125 I]IgG. In experiments A and B in which the tracer dose was given at the start of the drug treatment, no clear-cut conclusions could be made about an effect of methylprednisolone on the metabolic clearance of [125 I]IgG (data for experiment A in Fig. 6g, h, and i). This was because the injected isotope did not reach a steady state of catabolism before the end of drug treatment, and thus each individual's base-line clearance could not be established. In experiment C on the other hand, a steady state existed for 1 wk before administration of methylprednisolone (Fig. 8b). In this experiment, there was an increase in the rate of plasma clearance of [125 I]IgG in the three treated volunteers during the time the drug was administered whereas there was no change in the control volunteers. The increase in plasma clearance was evident within 24 h of the onset of drug therapy in two of the treated volunteers and within 48 h in the other. The mean [125 I]IgG plasma clearance for the three volunteers during the 3 days before drug treatment was a 2.71 ml plasma/24 h/kg; during drug treatment, the rate increased by 40% to a mean of 3.79 ml plasma/24 h/kg. Individual increases were 36, 43, and 51% of base line, respectively.

Specific activity of serum IgG. The specific activity of serum IgG (counts per minute/milligram) was measured daily during and after administration of methylprednisolone in the 12 volunteers in experiment A and the

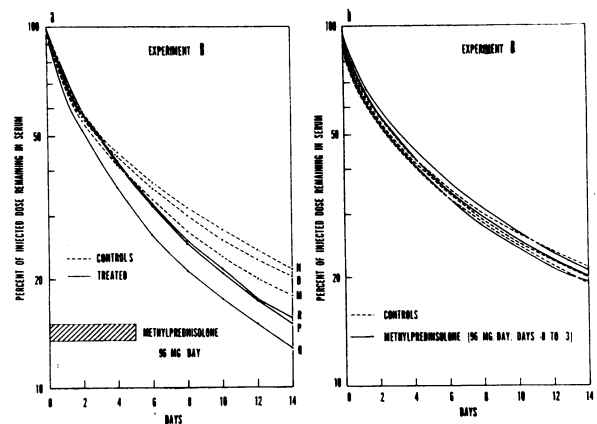


FIGURE 7 Decreased plasma survival of [125 I]IgG when the labeled protein is given at time of administration of methylprednisolone, (panel a) but not when given 3 days after completion of drug treatment (panel b). Code letters represent individual volunteers.

12 volunteers in experiment B. The data for experiment A are shown in detail in Fig. 9 and the serial specific activities are plotted in curves as percent of the zero-time value. The shapes of the curves of five of the eight treated volunteers were distinctly different from those of the four controls represented by the shaded area. Beginning about day 3, the curves tended to flatten indicating that the specific activities of IgG in the treated volunteers were higher than those in the controls. By comparing the data in Figs. 6 and 9, it can be ob-

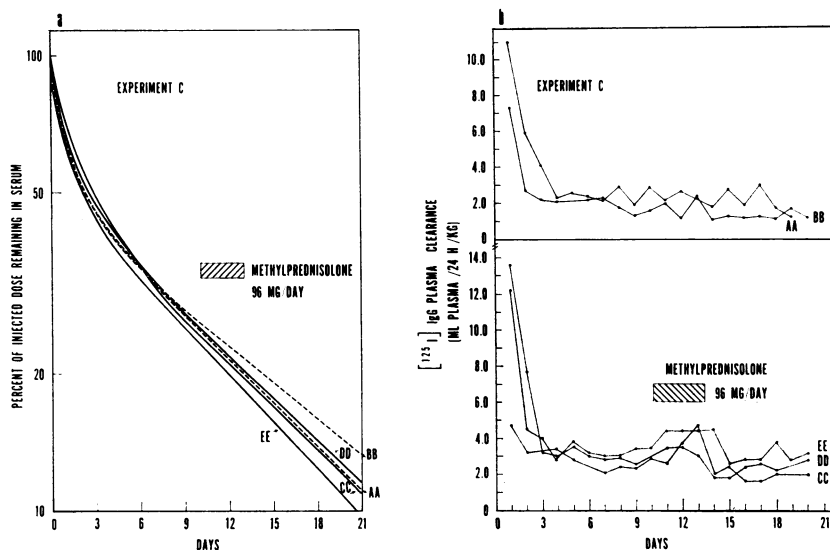


FIGURE 8 Increased metabolic clearance of [125 I]IgG during 3 day period of administration of methylprednisolone (panel b) without markedly altering plasma survival of IgG (panel a). The IgG concentrations on day 20 in the control volunteers AA and BB were unchanged from pretreatment values. Of the treated volunteers, CC had a decrease in IgG from 9.6 to 6.0, DD a decrease from 8.5 to 6.6 and EE a decrease from 9.6 to 5.9 mg/ml, respectively.

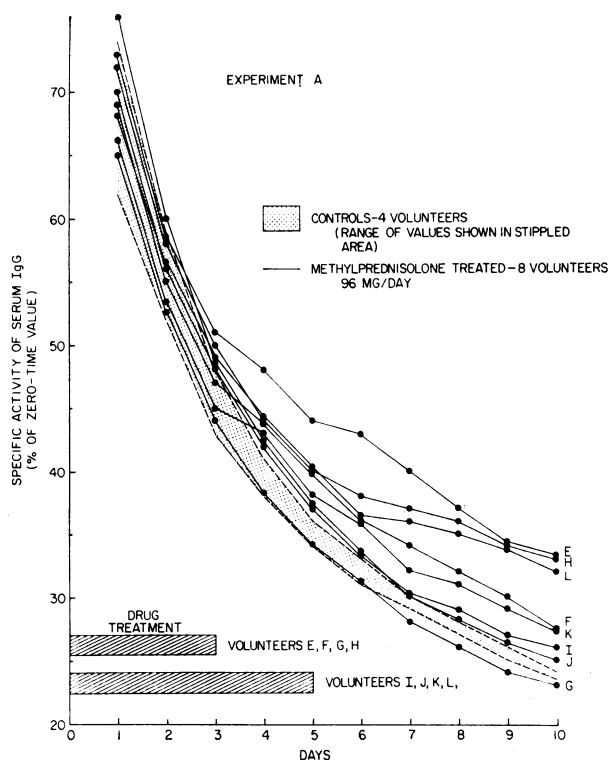


FIGURE 9 Decreased entry of newly synthesized IgG into the circulation of methylprednisolone-treated volunteers. The specific activities of IgG in the sera of four controls represented by the shaded area are compared with those of eight methylprednisolone-treated volunteers. [125 I]IgG was given on day 0. By comparing this figure with the data in Fig. 6, note that volunteers whose IgG concentrations decreased the most had relatively higher specific activities of IgG after drug treatment.

served that the onset of flattening of the curves often coincided with the onset of decreases in serum IgG concentrations. In contrast, the curves of two of the three

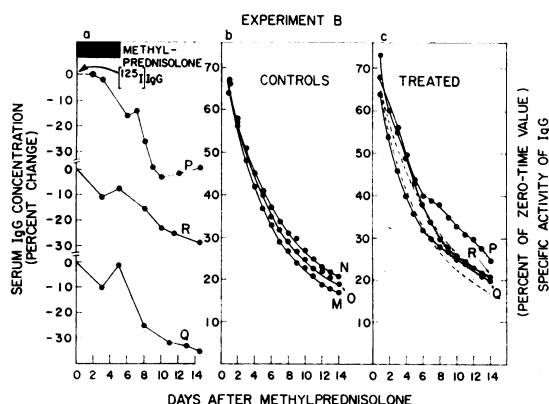


FIGURE 10 Specific activity of IgG in serum of three volunteers who received 5 days of methylprednisolone and in three volunteers who were untreated controls. The labeled IgG was given at the beginning of drug treatment.

remaining treated volunteers (I and J) were similar in shape to those of the controls, but the specific activities were also slightly higher than those in the controls. Note in Fig. 1 and Table II that these two volunteers had only minimal early and late changes in serum IgG concentrations. The decline in the specific activity of the [125 I]IgG in the plasma of volunteer G was similar to that seen in the untreated controls. He also showed the least change in IgG concentration among the drug-treated volunteers.

The specific activity data of the 12 volunteers in experiment B are shown in Figs. 10 and 11. The control volunteers (Figs. 10b and 11b) showed smooth decreases in the specific activities of their IgG which confirmed the findings in experiment A. In contrast and again confirming results of experiment A, the three volunteers who were given the labeled IgG on the 1st day of drug treatment showed flattening of their specific activity curves (Fig. 10c). The flattening was more pronounced in volunteers P and Q in whom the rate of fall of serum IgG concentration between days 5 and 10 was twice that seen in volunteer R.

Finally, when the labeled IgG was given 3 days after completion of a 5 day course of methylprednisolone, the shapes of the specific activity curves were nearly identical with those of the controls (Fig. 11c).

To evaluate whether the changes in specific activity of [125 I]IgG might be propelled by the same forces which caused the depression of the serum IgG levels, we examined whether there was a correlation between the specific activity of [125 I]IgG and the depression of levels of IgG in the serum of patients in experiments A and B. The analysis was done on the last day on which serial data was collected (day 10 in experiment A and day 14

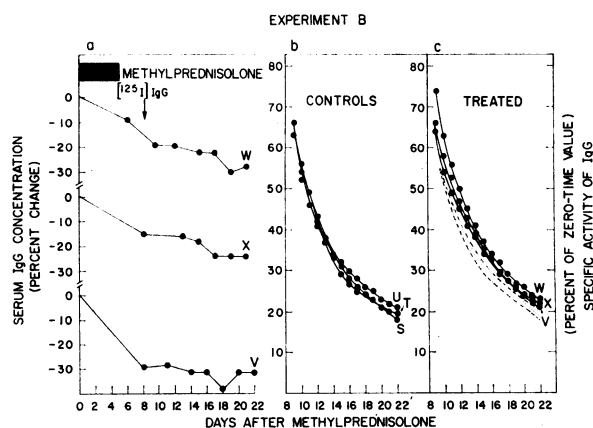


FIGURE 11 Specific activity of IgG in serum of three volunteers who received 5 days of methylprednisolone and in three untreated volunteers. The labeled IgG was given 3 days after completion of drug treatment. Note normal shapes of the decay curves of specific activity in the treated volunteers.

in experiment B). In both experiments, the least percentage decrease in specific activity of IgG was associated with the greater percentage decrease in serum IgG. (in experiment A: $r = 0.78$, $P = 0.001$ and in experiment B: $r = 0.93$, $P < 0.001$, Spearman rank correlation test [18]).

DISCUSSION

These results demonstrate that a 3 or 5 day course of 96 mg/day of methylprednisolone given in 6 divided doses caused sustained decreases in serum IgG in 12 (86%), in serum IgA in 6 (43%) and in serum IgM in only 2 (14%) of 14 treated volunteers. The decreases in serum IgG concentration began within several days after starting methylprednisolone. The rate of decrease slowed during the week after cessation of corticosteroid treatment, suggesting that recovery began shortly thereafter. The magnitude of the drug effect was dose-related: after 3 days of methylprednisolone, the lowest IgG levels were seen during the 2nd wk after treatment whereas after 5 days of drug, the lowest IgG levels occurred during the 3rd and 4th wk. Moreover, an upward trend in IgG concentration occurred earlier after the 3 day course as compared with the 5 day course of treatment; the rate of recovery of IgG serum levels was inversely related to the rate of fall due to the drug treatment. Considerable individual variation occurred in the magnitude of the effect of methylprednisolone on immunoglobulin concentrations, and in several volunteers, only minimal effects were noted. Whether or not these variations reflect differences in absorption, distribution, and metabolism of the drug or individual differences in cellular sensitivity to drug action (19) remains to be determined.

There are several mechanisms, or combinations of mechanisms, that can be evoked to explain the observed decreases in serum IgG: (a) we can exclude laboratory variation or technical error. The mean decrease in IgG concentration in 14 volunteers was minus 2.5 mg/ml (Table I), a value considerably greater than the error of measurement (See Methods). More importantly, however, control volunteers studied simultaneously under the same conditions except for administration of methylprednisolone showed no significant changes in IgG concentration (mean decrease in 10 control volunteers = 0.2 mg/ml). Finally, results of experiments carried out on three separate occasions were similar.

(b) Increased catabolism of IgG could account for the decrease in serum IgG. In the one experiment in which radiolabeled IgG was given 10 days before 3 days of drug treatment, methylprednisolone caused increases of 36–51% in the rate of plasma clearance of [125 I]IgG. However, this effect, which became evident by the 2nd day of drug treatment, disappeared within 48 h of stop-

ping methylprednisolone. Whether the effect was more pronounced or persisted longer in the volunteers treated for 5 days cannot be determined from our data. It must be noted, however, that decreased plasma survival of [125 I]IgG was seen in six of seven volunteers who received a 5 day course of drug begun at the time of tracer injection, whereas, it was seen in only one of the seven volunteers who received a 3 day course of methylprednisolone. If the decreased plasma survival reflects increased catabolism, then it would suggest that the 5 day course of drug caused a greater effect on catabolism than the 3 day course. It should be emphasized, however, that in the absence of a steady state, the measurement of half-life survival of IgG may not be a valid measure of the rate of catabolism. Nevertheless, we performed one experiment in which methylprednisolone was administered for 3–8 days before injection of the tracer dose and we found that the plasma survival was identical in treated and control volunteers (Fig. 7b). This strongly suggests that the decreased plasma survival of labeled IgG in the majority of volunteers who received 5 days of drug was a delayed manifestation of something that took place only during and for no more than 3 days after drug treatment. Thus, the observation noted above, that increased catabolism of IgG ceases within 2 days of stopping methylprednisolone and the findings that the drug-induced effect on plasma survival of IgG does not extend beyond 3 days after drug treatment, leads one to speculate that the decreased plasma survival of IgG is due principally to increased catabolism during the drug treatment period. Unfortunately, definitive proof for this hypothesis is lacking in our data since we did not perform experiments in which the fractional catabolic rate of labeled IgG had reached a steady state before administration of 5 days of methylprednisolone.

(c) It is possible that methylprednisolone caused alterations in the intra- and extravascular distribution of IgG. We cannot exclude this possibility for certainty since we did not measure the plasma pool serially throughout the study. Nevertheless, two pieces of data make this possibility seem unlikely as a significant cause of the decrease in serum IgG: (a) the decreases in IgG persisted for a long time after drug administration. Serum IgG levels were still decreased in 9 of 14 treated volunteers 28 days after drug treatment and in all 4 volunteers studied after 90 days. (b) If it were true that the methylprednisolone causes persistent changes in the distribution of IgG, then we should not have observed normal plasma survival curves in the three volunteers treated 3–8 days before the tracer study (Fig. 7b). Close examination of Fig. 1 reveals that the period of most rapid decrease in serum IgG concentrations in treated volunteers is that during the week immediately after drug treatment. Thus, since the [125 I]IgG was injected near the point of the

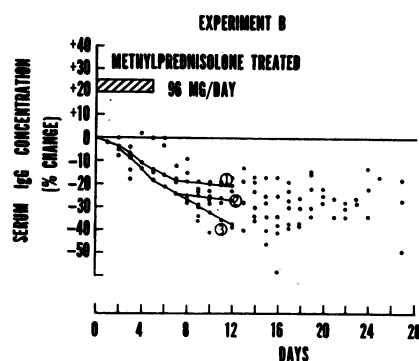


FIGURE 12 Theoretical changes in the total body pool of IgG compared with actual changes in serum IgG concentrations observed in experiment B. See Methods section for basis of calculations. Curve 1 represents condition of 50% inhibition of IgG synthesis for 5 days, curve 2 represents 100% inhibition for 5 days and curve 3 represents 100% inhibition for 10 days. In each case, it was assumed that catabolism increased by 50% during the drug treatment period, and that blood drawing caused the loss of 6 mg IgG/kg/day.

maximum rate of decrease in IgG in the three volunteers shown in Fig. 7b, the failure to detect any abnormality of plasma survival of the IgG is evidence against an extravascular shift of any great magnitude.

(d) An expansion of plasma volume alone cannot explain the persistent decreases in serum IgG in treated volunteers. As shown in Fig. 6, methylprednisolone caused significant increases in body weight, presumably due to fluid retention. However, these changes were transient and did not last beyond the 2nd wk. Moreover, characteristic weight changes occurred in two volunteers in the absence of changes in IgG concentration.

(e) External loss of IgG due to blood drawing certainly occurred. In most experiments, a maximum of 35 ml and a minimum of 5 ml plasma was removed each day. If the maximum amount of plasma had been withdrawn daily for the first 12 days, the body pool of IgG would have decreased by only 5.4% by the 12th day based on calculations and assumptions described below in Fig. 12. The fact that the control volunteers showed a mean net decrease in plasma IgG of only 1.3% suggests that under normal conditions, the IgG lost due to blood drawing is readily compensated for.

(f) The final major effect that methylprednisolone could exert is that of decreased synthesis of IgG. In support of this possibility are the following: (a) the lack of sufficient magnitude of the above effects to account for the decreases (b) the marked prolongation of the effect of a short course of drug and (c) the results of the experiments in which the specific activity of IgG was measured serially during and after administration of methylprednisolone. The shapes of the specific activity

decay curves were distinctly different from those of the controls and indicated that drug treatment caused the maintenance of relatively higher specific activities of IgG. This finding can be interpreted as follows: methylprednisolone treatment causes a decrease in the amount of newly synthesized IgG that enters the circulation. As a consequence, the labeled IgG in the circulation is not diluted with newly synthesized unlabeled IgG. Thus the IgG has a higher specific activity than is found in the absence of drug treatment.

From the above it is obvious that several factors may have been responsible for the decreased plasma concentrations of IgG. We therefore made several assumptions and then calculated whether the additive effects of methylprednisolone on increased catabolism, external loss, and decreased synthesis of IgG could reasonably account for the observed changes. Examination of Fig. 12 indicates that the observed decreases in plasma IgG concentrations are at least consistent with the postulated changes in the total body pool of IgG. For example curve 1 illustrates what would occur if there were a 50% decrease in IgG synthesis for 5 days resulting in a decrease in the total body pool of IgG equal to 223 mg/kg. 71 mg of this decrease would have been due to losses in blood drawing, 78 mg due to increased catabolism during treatment, and 74 mg due to decreased synthesis. On the other hand, curve 3 represents the situation of complete inhibition of IgG synthesis for 10 days. In this case, the total decrease in the IgG pool would have been 401 mg/kg, of which 72 mg would have been due to blood drawing, 80 mg due to increased catabolism, and 249 mg due to decreased synthesis.

An occasional volunteer had striking decreases in serum IgM and IgA concentrations early during the period of drug administration. We do not have any data to suggest a mechanism for these early changes, but it should be noted that decreased synthesis alone could not account for the magnitude of the changes (17). This would imply that in these cases, methylprednisolone may have exerted its major effects on catabolism or distribution of Ig among intra- and extravascular pools.

Previously, it has been observed that continuous administration of corticosteroids may lower serum gamma globulin or immunoglobulin concentrations in patients with striking hypergammaglobulinemia (20, 21), or in patients requiring prolonged therapy for chronic diseases (22-26). To our knowledge the present studies are the first to show that a short, limited course of methylprednisolone causes pronounced decreases in serum immunoglobulins which in some cases may persist for at least 90 days. This raises for discussion possible cellular mechanisms by which both the immediate and prolonged effects occur. It has been known for many years that lymphocytes are destroyed by corticosteroids (3-5) and re-

cent reports suggest that thymus-derived lymphocytes may be particularly susceptible to the effects of hydrocortisone (27-29). The fact that we observed a lag phase between drug administration and the time of the greatest fall in serum IgG would suggest that perhaps the major effect of methylprednisolone on immunoglobulin-forming cells is directed at a precursor cell rather than against the plasma cells that are actively secreting immunoglobulin at the time the drug is given.

It is not known whether this effect can be generalized to the entire class of corticosteroid hormones. Hydrocortisone for example has been shown to increase the catabolism of immunoglobulins in mice in addition to possibly inhibiting their synthesis (30). A recent study, in which eight patients were given 30 mg prednisone daily, suggested that this drug may also cause an increased catabolic rate of IgG (31). This conclusion may be unwarranted, however, since the authors utilized a method of analysis that assumes the presence of a steady state. On the other hand, cortisone acetate does not affect the catabolism of antibodies in the rabbit (32, 33).

A number of additional unknowns remain. We need to determine the effects of methylprednisolone on primary and secondary antibody formation, and particularly the effect of dose and timing of drug administration in the regulation of these processes. Nevertheless, certain therapeutic implications may be drawn on the basis of the present studies. Since it is evident that a short course of methylprednisolone causes prolonged decreases in circulating immunoglobulin, periodic pulsatile treatment with high doses of corticosteroids under certain circumstances, may be equally effective and perhaps a preferred method to reduce immunoglobulin production as compared with continuous treatment with lower doses. One might thereby be able to avoid the continuous suppression of general host-defense mechanisms and other vital metabolic processes, while, at the same time achieving the desired degree of inhibition of immunoglobulin synthesis.

ACKNOWLEDGMENTS

The authors thank Mr. Harold Summerlin and Mrs. Lucy Kormeier for technical assistance and Mrs. Marjorie Fulton, R.N., for coordinating the care of the volunteers while in the Clinical Research Center. We thank Mrs. Carol Dyess for assistance in preparation of the manuscript. Dr. George J. Beto, Mr. W. D. Kutach, Mr. Howard L. Sublett, and Mr. Vernon Floyd of the Texas Department of Corrections assisted in the volunteer program. The cooperation of the volunteers is to be commended.

This research was supported by U. S. Public Health Service, National Institutes of Health Research Grants HE 05435 and AM 15494, General Clinical Research Center Grant RR 00350, and by the Veterans Administration.

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