Factors Affecting Human Antibody Response

II. Effects of Large Doses of Homologous Gamma Globulin

ROBERT E. HODGES, M.D.,* WILLIAM B. BEAN, M.D.,† MARGARET A. OHLSON, PH.D.‡ AND ROBERTA E. BLEILER, PH.D.§

IN NATURE one encounters many instances of an animal's failure to produce a vital substance if this substance is supplied from outside sources. The most dramatic examples of this principle are furnished by the hormones, particularly thyroid, estrogens, androgens and adrenal cortical steroids. There is some indication that in man hemoglobin or serum albumin will not form if he is given a surfiet of these substances by transfusion, but the evidence is not complete.

Applying this line of reasoning we decided to determine whether massive parenteral doses given to healthy men would stop the production of gamma globulin, and hence antibodies. The safety of this procedure has been demonstrated in patients with malignant disease.[1]

METHODS

Six healthy volunteer subjects from a prison were housed on the Metabolic Ward of the University Hospitals. They were in good health, and ranged in age from twenty-three to thirty-three years. Three of them were given infusions and injections of gamma globulin, while the other three served as controls (Table I).

The quantity of gamma globulin given was based upon an estimate of the blood volume of each patient and the concentration of circulating gamma globulins as determined by electrophoresis. It was our aim to give approximately the same quantity which was being formed endogenously. We assumed that human gamma globulin had a "half-life" of one week. The quantities we gave approximated this rate of production (Table I).

Immunizations were given by injecting 0.5 ml. each of the commercial antigens of tetanus, typhoid and Asian influenza at the beginning of the third, fourth and fifth weeks of the study. All these men had previously been immunized against tetanus and typhoid both in military service and at the time of their admission to the prison.

TESTS AND PROCEDURES

The men were kept under continual observation. In addition to routine measurements of temperature, pulse, respiration and blood pressure, they were weighed daily. Each week a complete blood count and urinalysis was obtained. Blood was drawn each week for electrophoresis and for determinations of hematocrit, sedimentation rate (Table II), total nitrogen and zinc sulfate turbidity. Samples of serum were frozen at minus 60°F. for subsequent determinations of antibodies against tetanus, typhoid, Asian influenza and the isohemagglutinins against Landsteiner groups A or B. Aspirations of bone marrow (sternal puncture) were made four times at two week intervals during the study for differential counts (Table III).

RESULTS

During intravenous infusions of gamma globulin in 5 per cent dextrose, the three experi-

From the Department of Medicine, University Hospitals, Iowa City, Iowa.

* Associate Professor, Department of Internal Medicine; † Professor, Department of Internal Medicine; ‡ Professor, Department of Nutrition; § Associate Professor, Department of Nutrition, Present address: Animal Science Laboratory, University of Illinois, Urbana, Illinois.

This study was financed in part by grants from the U. S. Public Health Service (A-1408) and by grants from the National Vitamin Foundation.

Portions of this material have been presented at the Fifth International Congress on Nutrition, Washington, D. C., September 5, 1960, and at the Gordon Research Conferences on Food and Nutrition, August 23, 1961, New London, New Hampshire.

TABLE I
The Experimental Plan

Subject	Age (yr.)	Quantity of Gamma Globulin* Given in Four Weeks (cc.)
1	32	606
2	23	769
3	29	925
4	33	Placebo
5	26	Placebo
6	27	Placebo

* Gamma globulin was given as a 16 per cent solution (Lederle Human Immune Globulin lot No. 2175-110A). Five milliliters was given intramuscularly four days of each week and from 100 to 180 cc. in 1,000 ml. 5 per cent glucose given intravenously once weekly. Control subjects received identical injections of a colored placebo.

mental subjects complained of unpleasant symptoms similar to those reported by Moore et al.[1] The distress was most severe during the first infusion and became progressively less with each successive infusion. This consisted of headache, pain in the lumbar region of the back, chilly sensations, generalized aching, nausea and anorexia. There was no vomiting. Measurements of pulse rate, blood pressure and temperature during these periods showed no abnormalities. The control subjects had no complaints during their infusions.

Routine blood counts disclosed that initially several men had a mild anemia possibly related to the prison diet. This anemia disappeared during the period of observation. It was not related to their status as either control or experimental subjects. Hematocrit values did not reflect evidence of hemodilution as a result of gamma globulin infusions. Erythrocyte sedimentation rates, however, became faster in the three men who received gamma globulin (Table II). Routine examinations did not disclose proteinuria or abnormal formed elements in the urine at any time. The total concentrations of serum nitrogen remained unchanged or increased slightly. Measurements of the serum protein fractions showed increases of gamma globulin concentration in the three subjects given gamma globulin (Table IV) and (Fig. 1). Zinc turbidity increased significantly in one subject (subject 3), but not in the others.

Antibody titers initially reflected the previous immunizations these subjects had had against tetanus and typhoid (Table V). Typhoid antibody responses were found to be somewhat higher in the control subjects than in those who received gamma globulin (Fig. 2). The same was true for complement-fixing antibodies against Asian influenza, but not for hemagglutination inhibiting antibodies (Fig. 3). However, tetanus antibody titers, although somewhat higher in the experimental subjects (Fig. 4), were not significantly different in the two groups. Determinations of isohemagglutinin titers were too variable to allow interpretation.

TABLE II
Examinations of Peripheral Blood

Subject	Erythrocyte Sedimentation Rate (mm.)				Hematocrit (%)				Hemoglobin (gm./100 ml.)			
	Week				Week				Week			
	3	5	7	9	3	5	7	9	3	5	7	9
1	2	8	16	29	46	46	46	49	15.1	13.9	14.5	15.5
2	3	17	29	33	39	37	39	43	11.4	11.4	11.7	12.3
3	2	11	24	32	43	42	45	47	12.7	13.5	13.9	13.7
4	7	11	11	15	37	41	41	45	11.6	12.5	13.5	13.0
5	2	6	4	5	38	38	38	43	11.1	12.5	12.1	12.5
6	3	7	6	..	36	39	43	..	11.4	11.7	13.1	...

TABLE III
Bone Marrow

Subject	Blast Cells				Lymphoid				Erythroid				Myeloid				Monocytes				Eosinophils				Plasma Cells			
	Week				Week				Week				Week				Week				Week				Week			
	1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7
1	2.5	1.0	2.5	2.5	14.5	14.5	21.0	12.0	14.0	18.5	9.0	18.0	56.5	58.5	59.0	62.5	4.5	5.5	4.5	2.5	2.5	2.5	3.5	2.0	0.5	0.0	0.5	0.5
2	3.0	2.0	2.0	3.0	18.5	28.0	20.0	15.5	22.0	11.0	19.0	18.5	51.0	53.5	55.5	57.5	2.0	2.5	2.0	1.0	2.0	1.5	1.5	3.0	1.5	1.5	1.0	1.5
3	3.0	1.0	1.5	3.0	12.5	9.5	15.5	15.5	15.0	12.5	12.0	9.0	63.0	70.0	65.5	63.0	1.5	5.0	4.0	6.0	3.5	1.5	1.0	2.5	1.5	0.5	0.5	1.0
4	2.0	1.5	0.5	3.0	10.5	0.0	12.5	9.5	24.0	20.0	23.0	62.0	57.0	62.5	65.0	20.0	2.0	1.5	...	2.0	3.5	3.5	...	1.5	1.0	2.0	1.0	2.0
5	3.0	3.0	0.0*	2.0	11.5	16.0	40.0*	14.5	31.0	21.0	7.0*	17.0	46.5	51.0	47.0*	63.5	1.5	3.0	6.0*	2.0	6.0	5.0	...*	6.0	0.5	1.0	...*	1.0
6	2.0	1.0	0.0*	...	16.0	12.0	35.0*	...	18.5	15.0	1.0*	...	57.5	67.0	56.0*	...	5.0	2.0	7.0*	...	1.0	1.0	...*	...	0.0	2.0	1.0*	...

NOTE: Cell counts in per cent, ..., missing data.
* Diluted with peripheral blood.

TABLE IV
Serum Proteins

Subject	Zinc Turbidity (Units)							Total Serum Protein (gm./100 ml.)								Free Electrophoresis of Gamma Globulin (%)		
	Week							Week								Week		
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	1	7	10
1	8.0	6.0	3.0	5.7	5.8	5.3	5.5	7.15	7.50	7.75	7.31	7.12	7.19	7.69	7.00	5.3	13.7	13.8
2	4.2	6.8	3.8	7.8	7.5	7.8	8.3	7.56	7.81	7.62	7.62	8.06	7.50	8.25	7.75	10.8	19.9	15.6
3	5.7	8.4	6.7	10.3	10.9	12.1	12.0	7.25	7.62	7.62	7.69	7.06	7.94	8.12	8.31	9.1	24.6	17.0
4	7.0	11.5	5.8	8.0	7.7	7.6	7.4	7.56	7.88	7.81	8.25	8.31	7.38	8.25	7.56	12.4	10.5	...
5	4.8	7.0	5.1	5.3	5.0	4.6	4.5	7.31	7.31	8.25	7.38	7.50	6.69	7.75	7.56	9.8	11.5	10.3
6	5.4	8.1	6.1	8.3	7.1	6.9	...	7.06	7.88	7.69	7.62	8.12	7.75	7.62	8.06	14.4	12.0	...

TABLE V
Concentrations of Antibodies

Subject	Typhoid H* Week								Typhoid O* Week								Tetanus† Week							
	2	3	4	5	6	7	8		2	3	4	5	6	7	8		2	3	4	5	6	7	8	
1	160	160	160	80	160	5,000	5,000		10	5	5	5	10	80	160		0.6	0.7	0.8	1	6	26	28	
2	160	160	160	40	320	2,560	40,000		5	5	5	10	40	40	320		1.5	1.6	1.4	2	13	34	17	
3	80	80	80	160	640	2,560	40,000		5	5	5	10	20	40	160		1.5	1.5	1.4	3	17	10	16	
4	320	320	320	40	1,280	20,000	16,000		5	5	5	5	40	40	160		0.1	0.2	0.01	0.2	3	9	11	
5	320	160	160	160	1,280	2,560	80,000		5	5	5	5	80	80	640		0.3	0.4	0.3	0.6	3	11	11	
6	80	80	80	80	1,280	10,000	80,000		5	5	5	5	20	640	5,000		0.03	0.03	0.02	0.1	2	3	6	

* Typhoid O and H, reciprocal of title.
† Tetanus, mouse-protective units.

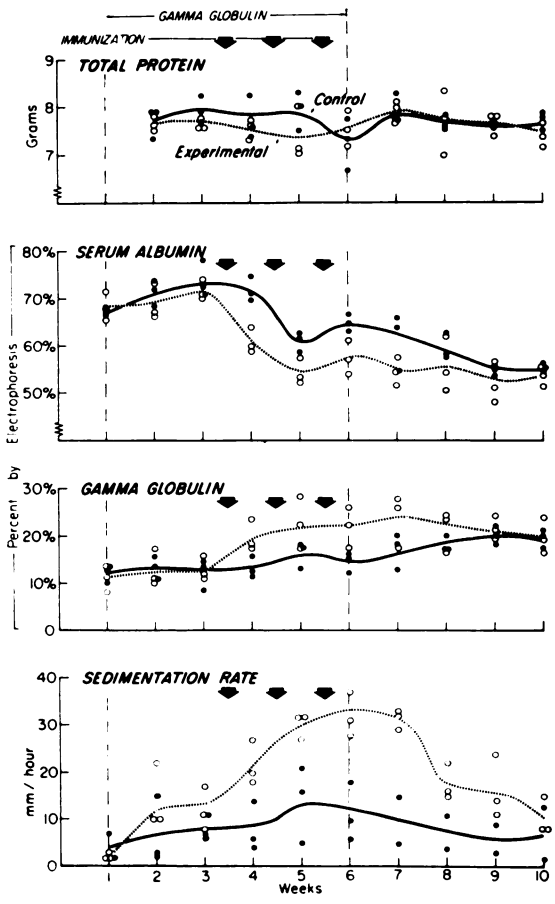

FIG. 1. Concentration in per cent of total protein, albumin and gamma globulin. As the concentration of gamma globulin increased the *relative per cent* of albumin decreased and the erythrocyte sedimentate rate increased.

COMMENTS

We sought to determine whether or not the administration of large quantities of human gamma globulin to normal men would (1) decrease formation of gamma globulin, and (2) inhibit antibody production. Interpretation of our results is difficult. If on e assumes that half of the circulating gamma globulin would be metabolized or lost in approximately seven days,[2,3] then the quantity which was given (calculated to equal amount produced) should have maintained, but not increased the concentration in the serum. Actually, the serum concentration doubled. Hence, one might assume that production was not affected. Several factors may alter this interpretation: (1) the

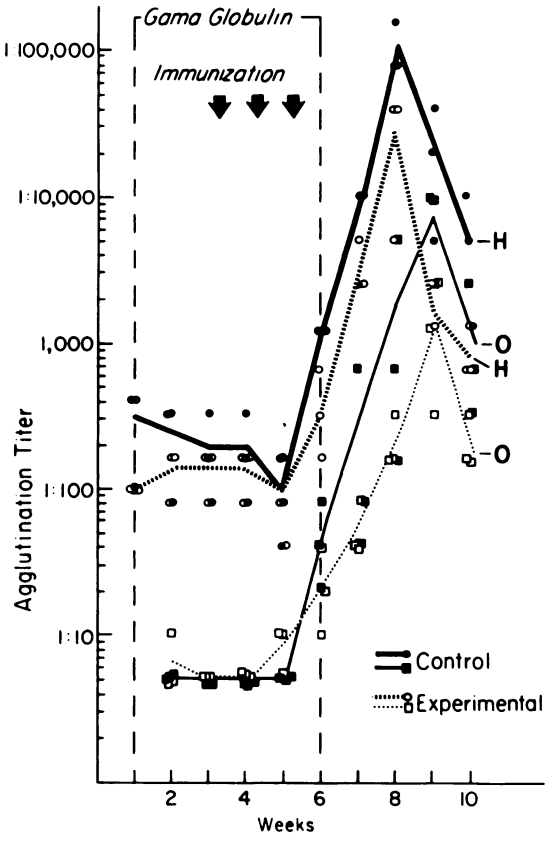

FIG. 2. Typhoid antibody responses were slightly lower in experimental subjects than in control subjects.

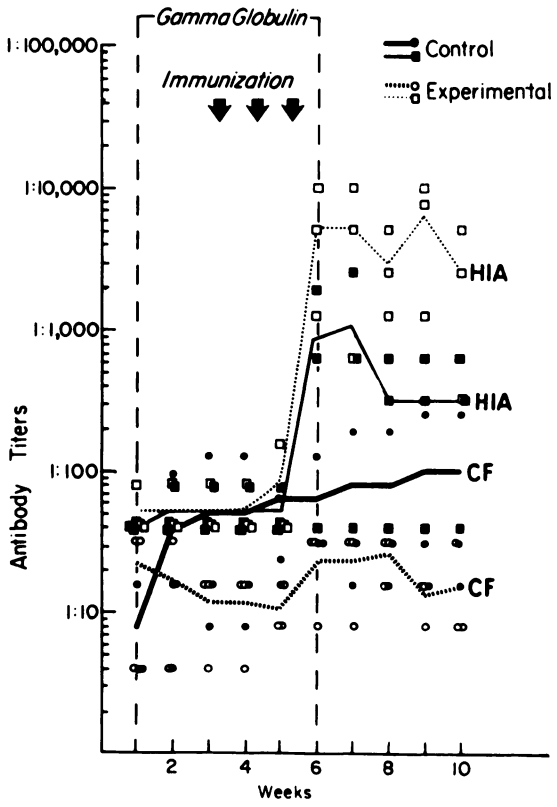

FIG. 3. Complement fixing antibodies against Asian influenza were lower in experimental than in control subjects. This was not true of hemagglution inhibiting antibodies.

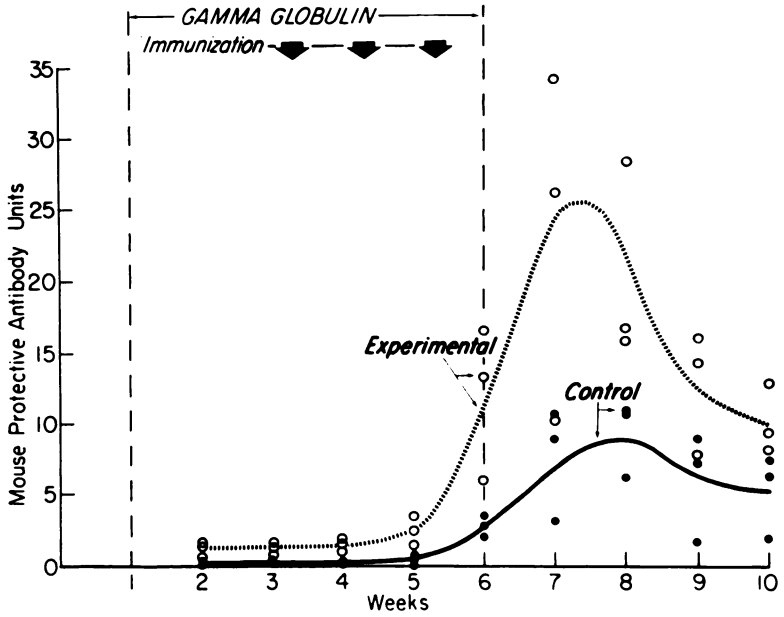

FIG. 4. Tetanus antibody response was not inhibited by gamma globulins.

quantity of gamma globulin in the body may be much more than that contained intravascularly, (2) the "half-life" of gamma globulin may be incorrect, and (3) if production of gamma globulin occurs in several different tissues (lymphocytes, plasma cells and possibly others), inhibition might be partial (affecting one source, but not all).

The evidence for inhibition of certain types of antibodies (typhoid H and O, complement-fixing antibodies against Asian influenza) in response to immunization is fairly good. Mouse protective antibodies against tetanus toxin, however, were not inhibited. Since this work was carried out, similar studies in animals have been reported.[4] Homologous gamma globulins inhibited both gamma globulin production and antibody production.

SUMMARY

The effect of administering large doses of human gamma globulin to three healthy men was studied. Three other men served as controls. Intravenous administration of gamma globulin was accompanied by unpleasant but harmless symptoms. Measurements of the concentration of gamma globulin in the serum showed that the amount was approximately doubled. The increase was accompan ed by an accelerated erythrocyte sedimentation rate. No toxic effects of this procedure could be detected in the renal, hepatic or hematopoietic systems. Plasma cells did not disappear from the bone marrow. The response of experimental subjects to tetanus antigens was normal; responses to typhoid H and O antigens and to Asian influenza antigens were less than those of control subjects.

ACKNOWLEDGMENT

We are indebted to Dr. Albert P. McKee, Department of Bacteriology, State University of Iowa, for some of these antibody determinations.

We also wish to express our thanks to Warden John Bennett of the Iowa State Prison, to the Board of Control of the State of Iowa, and to the prisoners who volunteered for these studies.

REFERENCES

1. Moore, G. E., Sandberg, A. and Amos, D. B. Experimental and clinical adventures with large doses of gamma and other globulins as anticancer agents. *Surgery*, 41: 972, 1957.
2. Lippincott, S. W., Korman, S., Fong, C., Stickley, E., Wolins, W. and Hughes, W. L. Turnover of labeled normal gamma globulin in multiple myeloma. *J. Clin. Invest.*, 39: 565, 1960.
3. Slater, R. J. and Sass-Kortsak, A. The turnover and circulation of the plasma proteins in the body. *Am. J. Med. Sc.*, 231: 669, 1956.
4. Trapani, I. L. and Campbell, D. H. Inhibition of antibody formation by homologous gamma globulin. *Fed. Proc.*, 19: 197, 1960.