TABLE I. Effect of Coagulase-Globulin on Residual Prothrombin Activity (Prothrombin Consumption) of Hemophilic Blood.

	No.	ml		ml	C.T., min	Min after formation of solid clot 15	30	45	60
						—Prothrombin activity, sec—			
1. N.W.B.		2			8	8	24	25	26
2. Hem. B.	1,	2			24	10	9	9	8
3.	1,	2	+ Saline,	.2	19	9	12	12.1	13.1
4.	1,	2	+ CG,	.2	7	9.8	16	16	16.6
5.	1,	1.8	+ CG,	.4	6	8.9	20	21.1	22
6.	1,	2	+ N.W.B.,	.2	14	10	13	13.5	14.5
7.	2,	2			135	12	11	11	11
8.	2,	2	+ Saline,	.2	110	10	12.9	15	15
9.	2,	2	+ CG,	.2	12	9.5	15	16	18
10.	2,	2	+ CG,	.4	9	10	17.5	18.5	20.1
11.	2,	2	+ N.W.B.,	.2	13	10	14.1	15.1	15.2

gen, and its deficiency in hemophilic blood suggests a specific deficiency of this plasma factor.

The observations that simple dilution of hemophilic blood with saline not only reduces its coagulation time in glass and in silicone tubes(1) but also alters the pattern of residual serum prothrombin activity (Table I, 3,8) have at the present time no ready explanation. Tocantins(5) has supposed that dilution disrupts an "accelerator-inhibitor complex" and that in hemophilia there is an excess of inhibitor, but the evidence for such an increase in inhibitor is not generally accepted. In any case, whether or not dilution disrupts normal or abnormal physico-chemical relationships, its effect is considerable, and it must be emphasized that great caution must be used in interpreting the results obtained from artificial systems, particularly in the *in vitro* assay of antihemophilic substances.

Summary. 1. The coagulase-globulin fraction of normal human plasma not only reduces the coagulation time of hemophilic blood but also alters the pattern of residual serum prothrombin activity in the direction of normal. 2. An equivalent amount of normal blood produces the same effect. 3. Simple dilution of hemophilic blood with saline by itself produces significant alterations in coagulation time and residual prothrombin.

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Experimental Hypersensitivity. Relationship of Dosage to Serological and Pathological Responses Following Injection of Heterologous Protein.* (19529)

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Although the histological response in the rabbit to injections of heterologous protein has been repeatedly described(1-5), the fre-

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quency of occurrence and the severity of the reaction has varied widely in these reports (6,7). An important variable has been the amount and type of protein used. As it is known that the frequency of serum sickness in humans is directly related to the amount of serum given(8), the same relationship might exist in the experimental model. Further, as the histological changes are evanescent and healing takes place promptly, the time of sacrifice would make uniform interpretation of the tissue response difficult. The purpose of this report is to determine how the amount of a uniform antigen would alter the serological and histological response in the rabbit.

Materials and methods. Animals. Male albino rabbits approximately 2 kg in weight were used in all experiments. *Serum protein.* Bovine serum gamma globulin (Lot Nos. C-1823B, C-188B and C-904) which had been prepared by the Armour Laboratories according to methods of Cohn and associates (9) was used as a 13.3% solution in 0.9% sodium chloride sterilized by Seitz filtration and used immediately. No preservative was added. *Sheep cells.* The sheep blood was drawn aseptically and preserved in modified Alsever's(10) solution. The cells were not used beyond the age of 6 weeks. When used, the cells were washed 3 times in the phosphate buffer, centrifuging at 2000 rpm for 10 minutes. Approximately 2% suspension of cells was made by adjusting the concentration so that 0.4 cc of the suspension when hemolysed with distilled water gave a reading of 700 on the Coleman Junior spectrophotometer at a wave length of 545 μ. This corresponds to a cell suspension of approximately 500000 cells per cu mm. *Diluent.* Phosphate buffer as described by Kent *et al.*(10) was used throughout. *Hemolysin.* Commercial hemolysin (Lederle, Lot Nos. 121A and 122A) prepared by the usual procedure was used. The optimal amount for sensitization of sheep cells was determined by titration as described by Kent(11). *Complement.* Blood was drawn from the marginal ear vein of the rabbit. This was spun in a refrigerated centrifuge at 0°C and the serum was removed and stored in paraffin-sealed tubes at minus 23°C until used. Complement titrations of all the

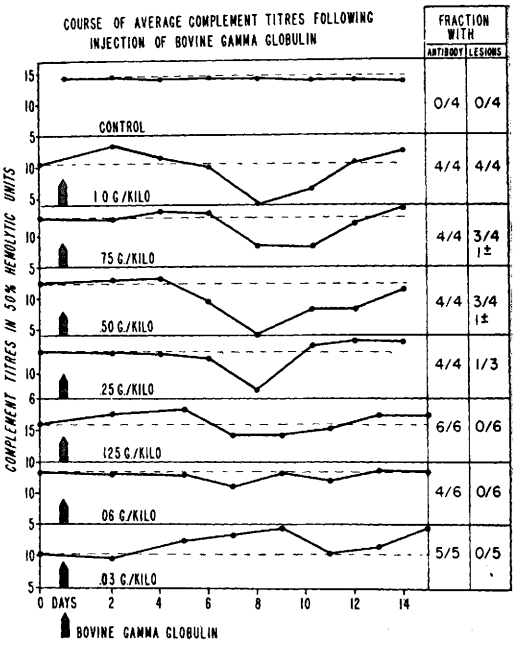

FIG. 1.

serum samples for a single animal were run at the same time. Titrations of complement modified for the Coleman Junior spectrophotometer were made as previously described(12). *Antibody.* Quantitative precipitin was obtained using the procedure of Heidelberger and Kabat(13,14) and the amount was determined by the ultraviolet absorption spectroscopy method of Gitlin(15). The extinction coefficients determined and used were 14.4 for rabbit antibody and 13.4 for the bovine gamma globulin antigen. When serum was sufficient the determinations were made in duplicate on 0.5 and .75 ml of serum; with insufficient serum for a satisfactory run, the ring test(1) was done. *Histological studies.* As in previous experiments, complete autopsies were made on the animals, but only the presence or absence of renal lesions are here recorded since it has been our experience that these histological changes are the most consistent of the several lesions attributable to experimental hypersensitivity.

Observations. Seven series of rabbits were given graded intravenous injections of bovine gamma globulin. The amounts of globulin were 1.0, .750, .500, .250, .125, .060, .030 g per kilo body weight. The complement titers

TABLE I. Effect of Graded Injections of Bovine Gamma Globulin on Time and Amount of Antibody Production.

Amount, g/kilo	Animal	Antibody in μg AbN/ml — Days after inj. 6	8	10	12	14	Renal lesion
1	28			++	18	18	Pos.
	30			19	++	++	"
	27				±	13	"
	29				12	18	"
.75	15			+	16	26	"
	21			12	14	++	Equivocal
	11				+	++	Pos.
	7				+	++	"
.50	25		±	25	20	++	"
	26			15	23	19	"
	23			11	+-+	18	Equivocal
	24				±	14	Pos.
.25	10		++	+++	+++	38	Neg.
	18		±	++	18	30	"
	8			+	14	18	Pos.
	19			8	11	27	Lost
.125	80	13	15	++	++	13	Neg.
	84		±	6	11	+	"
	85		8	15	14	++	"
	81			±	±	±	"
	82			±	±	+	"
	83				±	+	"
.06	88	11	—	++	—		"
	89	27	24	24	23	++	"
	86		11	12	15	+	"
	87			±	±	+	"
	90						"
	91						"
.03	93	15	30	26	18	12	"
	94		+++	+++	25	17	"
	97		±	±	±	++	"
	99		++	++	++	++	"
	95			++	++	+	"

and antigen or antibody were determined approximately every 2 days until sacrifice on the fourteenth day. From Fig. 1, it can be seen that the averages of the blood complement titers show a definite transient depression on about the eighth to tenth days in those groups given 1.0, .750, .500, and .250 g per kilo body weight. The averages tend to obscure the abrupt and striking fall in the serum complement in individual rabbits but even with the averages plotted, the fall is apparent only in those receiving the large amounts. When amounts given were .125, .060, and .030 g per kilo, the depression in complement is not regularly demonstrable in individual titrations and does not appear upon averaging the values.

Antibody formation occurred regularly in all animals except 2 in which neither antigen nor antibody were demonstrable after the ninth day following .060 g per kilo of the antigen. Table I illustrates the time and amount of circulating antibody found. In general, the smaller the amount of antigen injected, the earlier was the appearance of circulating antibody. Although there is great individual variation, the antibody nitrogen values showed no clear difference which could be related to the amount of antibody found and the presence or absence of histological changes.

The histological changes seen are recorded in Fig. 1. Renal lesions were frequent in the animals receiving larger amounts of antigen but were less common as the amount of antigen was reduced. The lesions could be seen nearly regularly in animals given 1.0, .750, and .500 g per kilo, in one animal given .250 g, and

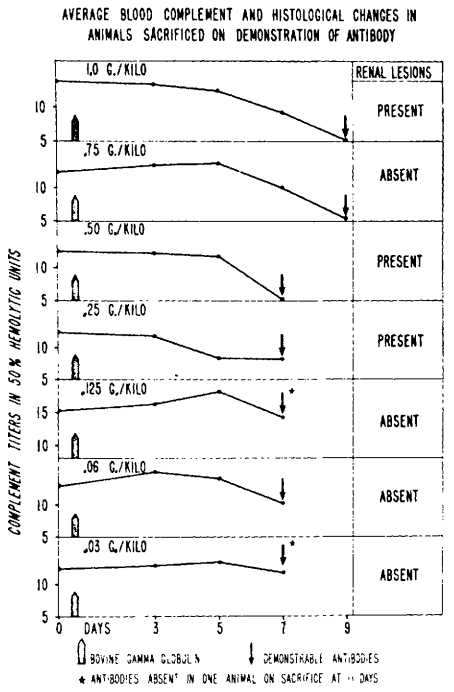

FIG. 2.

none in the animals given less than this amount. As the lesions are evanescent and because in those receiving a smaller injection antibody formation could be detected early, two animals in each group were sacrificed on the first or second day after appearance of demonstrable antibody in the circulating blood. The results of this study are seen in Fig. 2. Again renal lesions were seen in the more heavily injected group and no change attributable to hypersensitivity could be seen in those given less than .250 g per kilo body weight.

Antibody formation was always demonstrable in those animals which developed lesions and all such animals showed a depression of the circulating complement just at the time of the appearance of circulating antibody. The precipitin could be demonstrated when the complement was at its lowest level or immediately thereafter, as can be seen most clearly in Fig. 2. However, although the drop in complement was always associated with appearance of precipitins, its depression also occurred sporadically in those rabbits in which renal lesions were not demonstrable.

Discussion. Reports of the regular production of histological changes following the injection of heterologous protein have all involved the use of large quantities of antigen. More and Waugh(4) used 1.0 g per kilo of bovine gamma globulin. Hawn and Janeway (1) gave the same amount of bovine gamma globulin and bovine albumin. Rich and Gregory(3) used 10 ml per kilo of horse serum. Ehrich(2) *et al.* gave both 10 ml and 20 ml horse serum per kilo and observed that the larger dose was more effective. Further, it has also been shown that the specific protein used alters the frequency and location of histological changes(1).

From the work presented herein, it would appear that the amount of antigen injected directly influences the incidence of the renal lesions seen, and these changes are not related to the level of circulating antibody produced. This supports the observation of Rich and his coworkers(16) who found no correlation between the antibody titers and the appearance of lesions, but in contrast to Cohen *et al.* (17) who felt that the lesions were a function of antibody rather than antigen levels.

The depression of circulating blood complement occurring at the period when histological changes are most acute may represent its utilization in the interaction of antigen and antibody(12). This depression was most clearly evident in the animals receiving the larger amounts of antigen. Moderate declines were occasionally observed in some of the animals receiving the smaller amounts of antigen and these declines were regularly followed by the appearance of precipitins but without histological alterations.

From the evidence presented, the speculation is warranted that only when large amounts of antigen are used is the interaction of antigen and antibody prolonged and of such magnitude that the interaction is severe enough to produce histological changes and depletion of complement is detectable. The level of the circulating antibody may be merely an index of the amount of antibody in excess of that needed for neutralization of the antigen circulating and in the tissue, and may bear no direct relation to the histo-

logical changes seen.

Summary. Forty-seven rabbits were given graded injections of bovine gamma globulin intravenously. These were bled frequently for antibody and complement studies and then sacrificed for histological examination. When amounts used were from .250 g to 1 g per kilo body weight, glomerular lesions attributable to hypersensitivity were seen in most animals, and regular depression of complement followed by the appearance of circulating antibodies was demonstrable. In smaller doses, no histological changes occurred and the serological alterations were inconstant.

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Induction of Necrosis in Mouse Mammary Carcinoma by Cortisone.* (19530)

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The use of 11 dehydro 17 oxycorticosterone (Cortisone) in attempts to produce regression of neoplasms is not new. Heilman and Kendall(1) reported temporary regression of a mesenchymal tumor in mice after administration of this agent in doses smaller than those used in the present study. They did not report on the nature of the damage to the neoplasms. Vrat(2) has reported that cortisone has no effect upon mouse mammary carcinoma. The dose levels he used were smaller than those employed in our studies. There has been extensive clinical use of cortisone in the treatment of various types of neoplasms in man. The results have been disappointing as to long-term effects in spite of evidence of temporary remissions.

There is a large literature(3) on the use of bacterial toxins, colloidal substances of numerous sorts and other toxic agents in producing intra-tumoral hemorrhage and necrosis in man and other animals. In general it can be said that numerous such agents induce injury to neoplasms, greater in extent and degree than that inflicted on the host, but that no agent is known which regularly causes total

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