

THE IN VIVO FORMATION AND FATE OF ANTIGEN-ANTIBODY
COMPLEXES FORMED BY FRAGMENTS AND POLYPEPTIDE
CHAINS OF RABBIT γ G-ANTIBODIES*

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In vivo interaction of specific antibodies with circulating protein antigens leads to the formation of antigen-antibody complexes and both antigen and antibody are subsequently removed from the circulation (1). Complexes formed near equivalence or in antibody excess are very rapidly eliminated from the circulation, whereas complexes formed in far antigen excess persist in the circulation for a prolonged period of time, but are eliminated faster than either antigen or antibody when injected alone (2).

Recent studies on the structure of γ G-immunoglobulins have shown that complexes can be formed in vitro with certain fragments and polypeptide chains of γ G-antibodies. Fab fragments which contain only one antibody-combining site (3) form soluble complexes with antigen, whereas F(ab')₂ fragments which contain two antibody-combining sites form insoluble complexes with antigen (4). The formation of soluble complexes between antigen and specific H chains has been demonstrated (5-9), whereas conflicting findings have been reported concerning complex formation involving L chains (5, 8-10).

It is of both theoretical and practical interests whether complexes between antigen and antibody subunits are formed in vivo. The present experiments have been designed to study the in vivo formation and subsequent fate of complexes formed between a protein antigen and the fragments and the polypeptide chains of specific rabbit γ G-antibodies. It has been previously demonstrated that in the absence of specific antigen, fragments and polypeptide chains of γ G-immunoglobulins which lack the Fc portion of the H chain are rapidly eliminated from the circulation and catabolized (11, 12). In the present experiments γ G-subunits were injected into either rabbits or guinea pigs immediately following the injection of specific antigen. To follow the formation and in vivo fate of complexes, either the antigen or the γ G-subunit prepared from purified antibodies was labeled with I^{131} and their elimination from the circulation followed.

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Materials and Methods

Animals.—New Zealand rabbits, weighing approximately 2 kg and guinea pigs weighing 500 g were used in the experiments. All animals were fed Purina chow and water containing KI.

Antigens.—Crystalline bovine serum albumin (BSA), lot X69508, was obtained from Armour Pharmaceutical Co., Kankakee, Illinois. Guinea pig serum albumin (GpSA) was prepared from normal guinea pig serum according to a method previously described (13).

Antisera.—Anti-bovine serum albumin (anti-BSA) and anti-guinea pig serum albumin (anti-GpSA) was obtained from rabbits given several series of injections of BSA or GpSA, each series totaling 180 mg. The rabbits were bled 7 days after the last injection and their sera pooled.

Isolation of γ G.—The γ G-globulin fraction of rabbit antisera or normal rabbit serum was isolated by combined ammonium sulfate precipitation and DEAE-cellulose chromatography as previously described (11).

Purification of Antibodies.—Purified anti-BSA and anti-GpSA antibodies were prepared from the γ G-fraction according to the method described by Weliky et al. (14). 90 to 95% of the protein representing the purified antibodies was precipitated with the corresponding antigen. The in vivo behavior of purified anti-BSA antibodies was similar to the anti-BSA γ G-preparation obtained by DEAE-cellulose chromatography. Purified I^{131} -labeled antibodies injected into rabbits in the absence of antigen were eliminated from the circulation and catabolized at a similar rate as nonantibody γ G-preparations. I^{131} -labeled BSA injected intravenously into rabbits was rapidly and completely eliminated from the circulation after a subsequent injection of 1.3 times the equivalence amount of purified anti-BSA.

Labeling with I^{131} .—5 mg aliquots of either BSA, γ G, Fab, or $F(ab')_2$ fragments were labeled with I^{131} (I^*) according to the method described by McConahey and Dixon (15).

Papain and Pepsin Digestion of γ G.— γ G and purified antibodies were digested with twice crystallized papain (Worthington Biochemical Corporation, Freehold, New Jersey) according to the method described by Porter (3), except that the duration of digestion was limited to 4 hr. Fab fragments were isolated by carboxymethylcellulose chromatography, using 0.01 M, pH 5.5, acetate buffer (3). Pepsin digestion of either γ G or purified antibodies was performed with twice crystallized pepsin (Worthington Biochemical Corporation) according to the method described by Nisonoff et al. (4). Fifty-four to 58% of the original anti-BSA precipitating activity was recovered in the pepsin digest.

Preparation of H and L Chains.—H and L chains were isolated from purified anti-BSA or nonspecific γ G according to the method described by Fleischman et al. (16) using 0.15 M mercaptoethylamine and 0.2 M iodoacetamide for the reduction and alkylation procedures. After isolation by Sephadex G-75 gel filtration in 1 N propionic acid, the H and L chains were concentrated by pressure dialysis and dialyzed against phosphate-buffered saline pH 7.0. The concentration of the H chains was kept below 200 μ g per ml pH 7.0 buffered saline because of their low solubility at neutral pH. Examination of isolated H and L chain preparations by starch gel electrophoresis in 8 M urea at pH 2.9 (17) did not reveal any intact γ G in the H and L chain preparations. In all instances when I^* H and L chains were used, the purified anti-BSA or nonspecific γ G-preparations were first labeled with I^* , followed by the isolation of H and L chains. Seventy-one to 73% of the I^* activity was recovered in the H chain fraction and 27 to 29% in the L chain fraction.

Preparation of Hybrid Molecules.—Hybrid γ G-molecules were prepared according to the method described by Olins and Edelman (18). Equal amounts of I^* -labeled purified anti-BSA and I^* -labeled nonspecific rabbit γ G were reduced, alkylated, and their H and L chains separated. The H and L chain fractions were divided into two equal parts while still in propionic acid. Four mixtures were made of one part H chains and one part L chains; e.g. specific H chains plus specific L chains; specific H chains plus nonspecific L chains; nonspecific H

chains plus specific L chains and nonspecific H and L chains. The mixtures were subsequently concentrated by pressure dialysis and dialyzed against phosphate-buffered saline pH 7.0. A slight precipitate which formed after dialysis was removed by centrifugation. The preparations containing hybrid γ G-molecules were subsequently digested with papain and the Fab fragments isolated.

Preparation of Fd Fragments.—Fd fragments were isolated from reduced and alkylated I* labeled Fab fragments by Sephadex G-75 gel filtration in 1 N propionic acid as described by Fleischman et al. (5). The ascending portion of the first major protein peak representing dimerized Fd fragments and the descending portion of the second protein peak representing L chains were used in the experiments. The concentration of the Fd fragments was kept below 100 μ g per ml pH 7.0 buffered saline because of their relatively low solubility at neutral pH. No contamination of the Fd fragments with L chains could be demonstrated by Ouchterlony analysis with a guinea pig antiserum to rabbit L chains.

Elimination from the Circulation.—I* labeled or unlabeled BSA was injected intravenously into rabbits followed 5 min later by the injection of either labeled or unlabeled γ G or γ G-subunits. 1 mg of I* labeled γ G-subunits were injected into guinea pigs by cardiac puncture. The elimination from the circulation of either I* labeled antigen or γ G-subunits was determined as previously described (11). The elimination curves shown in Figs. 1 to 7 represent an average of determinations from data obtained from 6 to 8 animals.

Analysis of Antisera.—Rabbit anti-BSA and anti-GpSA γ G-antibody preparations were analyzed for precipitating antibody using a quantitative precipitin technique employing I* antigen (19). Purified antibody preparations were analyzed by the quantitative precipitin analysis technique described by Heidelberger and Kendall (20). Protein nitrogen determinations were performed by a modification of the micro-Kjeldahl technique using a Technicon auto-analyzer (21).

RESULTS

Effect of γ G-Fragments of Anti-BSA on Elimination of BSA.—Rabbits were injected intravenously with 2 mg I*BSA followed 5 min later by 12 mg anti-BSA γ G (1.1 times equivalence) or 12 mg anti-BSA digested with either papain or pepsin and the elimination from the circulation of the I*BSA was followed. As expected, the Fab preparation did not precipitate BSA in vitro, whereas the F (ab')₂ preparation retained 58% of its original precipitating capacity. As shown in Fig. 1, the I*BSA was completely eliminated from the circulation after injection of γ G-anti-BSA. Five of 6 rabbits showed a mild anaphylactic reaction 5 to 15 min after injection of anti-BSA. Injection of either Fab or F (ab')₂ fragments did not cause an immune elimination of I*BSA and none of the rabbits showed signs of anaphylaxis. When compared with control rabbits receiving only I*BSA, significantly more I*BSA remained in the intravascular compartment in rabbits injected with either anti-BSA Fab or F (ab')₂ fragments. The I*BSA was eliminated after initial equilibration at an exponential rate with a half-life of 4.5 to 5.0 days both in control rabbits and in rabbits receiving anti-BSA Fab and F (ab')₂ fragments. Most of the rabbits which received anti-BSA Fab and F (ab')₂ fragments showed an immune elimination of the BSA 7 to 10 days after injection, whereas control rabbits injected with only BSA showed an immune elimination 10 to 14 days after injection of the I*BSA.

The effect of increasing amounts of Fab and F (ab')₂ fragments on the elim-

ination of 2 mg I*BSA was studied by injecting fragments which were prepared from 24 and 48 mg anti-BSA γ G (2.2 and 4.4 times equivalence). The preparations of F(ab')₂ fragments prepared from 24 and 48 mg anti-BSA completely precipitated 2 mg of I*-labeled BSA in vitro, whereas the Fab preparations had no precipitating activity. The elimination curves of I*BSA after injection of these amounts of Fab and F(ab')₂ fragments is shown in Fig. 2. Injection of Fab fragments obtained from 24 and 48 mg anti-BSA did not cause an immune elimination of the I*BSA and again more I*BSA remained in the intravascular

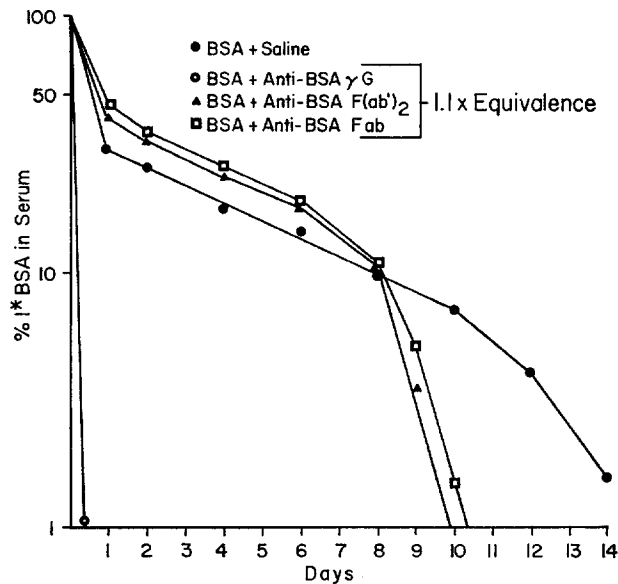


FIG. 1. Effect of 1.1 times equivalence γ G-anti-BSA and F(ab')₂ and Fab fragments obtained from 1.1 times equivalence anti-BSA on the elimination of I*-labeled BSA from the circulation of rabbits.

compartment as compared to control rabbits receiving only I*BSA. The rate of elimination of I*BSA after initial equilibration was similar to the rate of elimination of I*BSA in control rabbits. In contrast, F(ab')₂ fragments obtained from 24 and 48 mg anti-BSA caused an accelerated elimination of the I*BSA. The rate of elimination of I*BSA, however, was slower than that observed after injection of intact γ G-antibody and not all of the I*BSA was eliminated from the circulation. The I*BSA was eliminated after injection of 24 mg γ G-anti-BSA with a half-life of approximately 1 min. The elimination curves of the I*BSA during the first 60 min after injection of F(ab')₂ fragments obtained from 24 and 48 mg anti-BSA indicated that the complexes formed between I*BSA and F(ab')₂ fragments were heterogenous in respect to their rate of elimination. Some complexes might have been eliminated as fast as complexes formed by intact

γ G, whereas others appeared to be more slowly eliminated or not eliminated at all. In any event, more complexes were rapidly eliminated with the 48 mg dose than with the 24 mg dose of F (ab')₂ fragments.

Elimination of Fab and F (ab')₂ Fragments of Purified Anti-BSA in Presence and Absence of BSA.—The finding that more I*BSA remained in the intravascular compartment in rabbits injected with Fab or F (ab')₂ fragments (in slight antigen excess) compared to rabbits injected with only BSA suggested that the anti-BSA fragments formed complexes in vivo with BSA which persisted in the

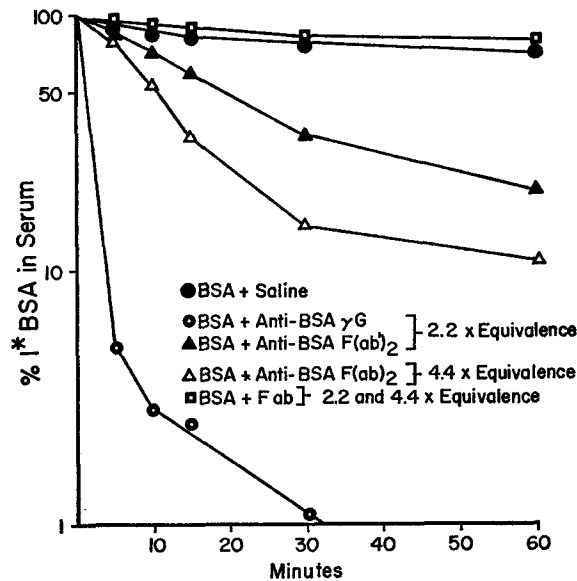


FIG. 2. Effect of 2.2 times equivalence γ G-anti-BSA and F(ab')₂ and Fab fragments obtained from 2.2 and 4.4 times equivalence anti-BSA on the elimination of I*-labeled BSA from the circulation of rabbits.

circulation. I*-labeled Fab and F (ab')₂ fragments of purified anti-BSA were, therefore, injected into rabbits which first received intravenous injections of either 500 mg BSA or the same volume of 0.15 M NaCl (Fig. 3). Fab and F (ab')₂ fragments were rapidly eliminated from the circulation in the absence of BSA. In the presence of BSA, anti-BSA Fab, and F (ab')₂ fragments remained in the circulation for long periods of time. After initial equilibration they were eliminated at an exponential rate with a half-life of approximately 5 days. The I*-labeled Fab and F (ab')₂ fragments were more rapidly eliminated 8 to 12 days after injection as a result of the immune elimination of BSA by the host.

Elimination of H and L Chains of Purified Anti-BSA in Presence and Absence of BSA.—Preparations of H and L chains isolated from 10 mg I*-labeled purified anti-BSA were injected into rabbits which previously received an intra-

venous injection of either 500 mg BSA or an equal volume of 0.15 M NaCl (Fig. 4). In the absence of BSA, the H chains, after initial equilibration, were slowly eliminated, whereas the L chains were very rapidly eliminated. In the presence of BSA, slightly more H chains remained in the intravascular compartment as compared to H chains injected into control rabbits and 8 to 12 days after injection, the I* H chains were more rapidly eliminated as a result of the immune elimination of the BSA by the host. The L chains were rapidly and similarly eliminated both in rabbits receiving BSA and in control rabbits receiving no BSA. Even the rate of elimination which occurred as early as 5 to

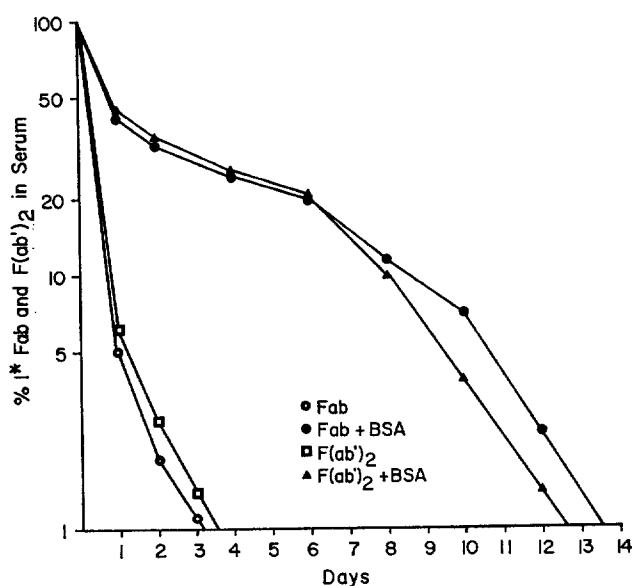


FIG. 3. Elimination of I*-labeled Fab and F(ab)₂ fragments of purified anti-BSA from the circulation of rabbits receiving 500 mg BSA and rabbits receiving no BSA.

30 min following injection was similar in both presence and absence of BSA. Incubation of I*-labeled H and L chains for 24 hr with BSA prior to the injection resulted in similar elimination curves. Again, no difference between the rate of elimination of the L chains in presence and absence of BSA was observed.

To determine whether excess specific H chains would cause a rapid elimination of BSA, 0.15 mg I*BSA was incubated in vitro for 4 hr at pH 7.0 with either 15 ml saline or 15 ml saline containing either 1.5 mg H chains or 3.0 mg H chains obtained from purified anti-BSA. The mixtures were subsequently injected into rabbits and the elimination of the I*BSA followed. The I*BSA which had been incubated with specific H chains was slowly eliminated from the circulation, similar to I*BSA which had been incubated with saline. Slightly

more I*BSA incubated with H chains remained in the intravascular spaces as compared to I*BSA incubated with saline. Similar findings were obtained in rabbits injected with 0.15 mg I*BSA followed 5 min later by injections of 1.5 or 3.0 mg isolated H chains of purified anti-BSA.

Elimination of Fd Fragments of Anti-BSA in Presence and Absence of BSA.—Fd fragments and L chains prepared from I*-labeled Fab fragments of purified anti-BSA were injected into rabbits which previously received either 500 mg BSA or an equal volume of 0.15 M NaCl (Fig. 5). In the absence of BSA both

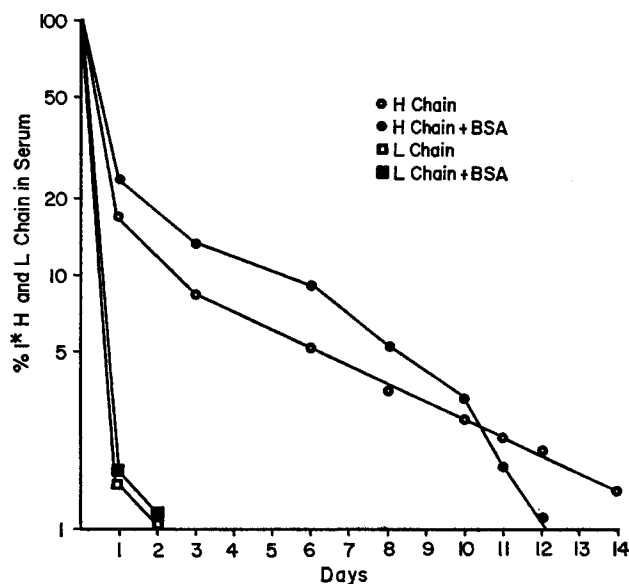


FIG. 4. Elimination of I*-labeled H and L chains of purified anti-BSA from the circulation of rabbits receiving 500 mg BSA and rabbits receiving no BSA.

Fd fragments and L chains were rapidly eliminated, while in the presence of BSA Fd fragments remained in the circulation and were eliminated with a half-life of approximately 5 days. The Fd fragments were more rapidly eliminated 9 to 12 days after injection as a result of an immune response of the host to the BSA. L chains prepared from Fab fragments were rapidly eliminated both in presence and absence of BSA.

Elimination of Hybrid Fab Fragments in Presence and Absence of BSA.—In order to determine the contribution of specific L chains and Fd fragments in the binding of the Fab fragments to the circulating BSA, Fab fragments obtained from hybrid γ G-molecules, containing either H or L chains from specific anti-BSA and the corresponding chain from nonspecific γ G were injected into rabbits previously injected with 500 mg BSA (Fig. 6). In the absence of BSA all preparations of hybrid Fab fragments were rapidly eliminated. In the

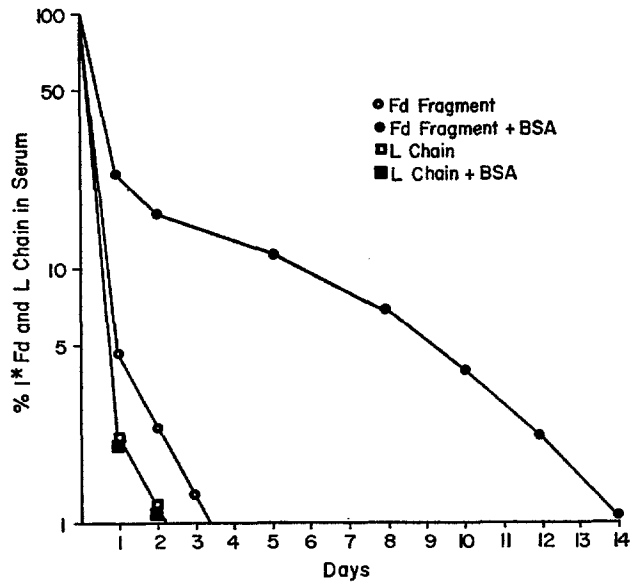


FIG. 5. Elimination of I^* -labeled Fd fragments and L chains of purified anti-BSA from the circulation of rabbits receiving 500 mg BSA and rabbits receiving no BSA.

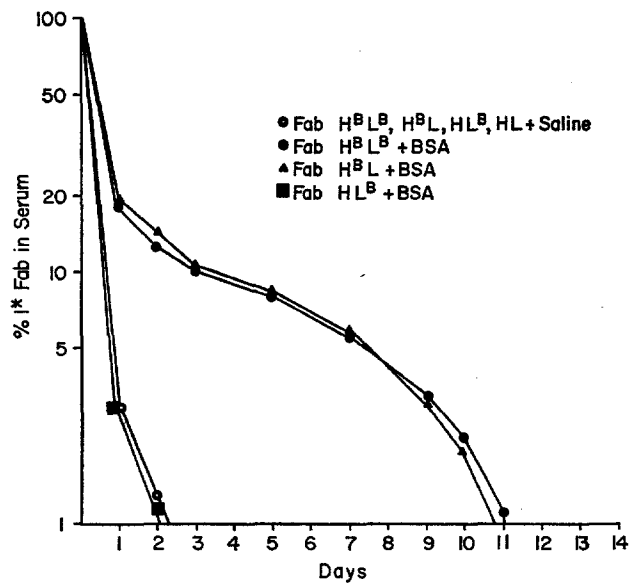


FIG. 6. Elimination of I^* -labeled Fab hybrids from the circulation of rabbits receiving 500 mg BSA and rabbits receiving no BSA. H^B , L^B = H or L chains derived from purified anti-BSA; H, L = H or L chains derived from nonspecific rabbit γG .

presence of BSA, Fab hybrids containing a specific Fd fragment and either a specific or a nonspecific L chain remained in the circulation with the BSA. In contrast, Fab hybrids containing a specific L chain and a nonspecific Fd fragment were rapidly eliminated in a similar manner as the Fab hybrids containing both nonspecific Fd fragments and L chain. Less of the Fab hybrids containing a specific Fd fragment remained in the circulation (Fig. 6) in presence of BSA as compared to untreated Fab fragments (Fig. 3). The elimination

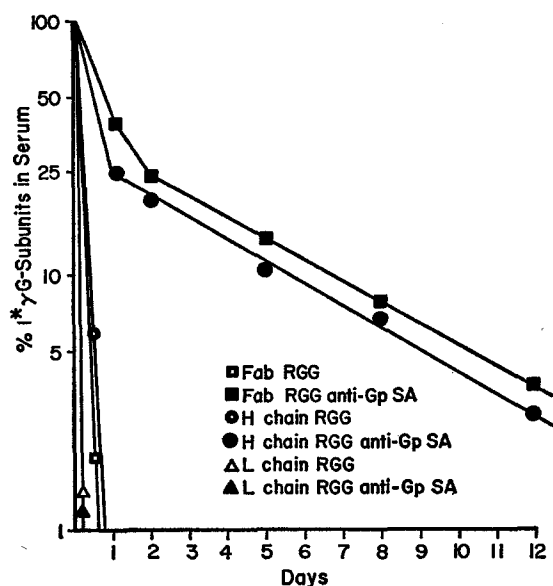


FIG. 7. Elimination of I*-labeled Fab fragments, H and L chains of purified rabbit anti-GpSA and of nonspecific rabbit γ G from the circulation of guinea pigs.

from the circulation of specific Fab fragments which had been reduced and alkylated only and Fab fragments which had been dialyzed against propionic acid after reduction and alkylation was therefore studied and compared with the elimination of untreated Fab fragments. In the presence of BSA, reduced and alkylated Fab fragments (but not dialyzed against propionic acid) were eliminated in a similar manner as untreated Fab fragments. However, following dialysis of the reduced and alkylated Fab fragments against propionic acid they were eliminated similarly to Fab hybrids containing a specific Fd fragment.

Elimination of γ G-Subunits of both Purified Rabbit Anti-GpSA and Nonspecific Rabbit γ G in Guinea Pigs.—Since no complex formation between anti-BSA L chains and BSA was observed in rabbits, L chains of purified anti-GpSA

were injected into guinea pigs in order to have a maximum *in vivo* concentration of antigen. Fab fragments and H chains of purified anti-GpSA and non-specific rabbit γ G also were injected into guinea pigs (Fig. 7). Fab fragments, H and L chains obtained from non-specific rabbit γ G were rapidly eliminated from the circulation of guinea pigs. Fab fragments and H chains of purified anti-GpSA remained in the circulation and were catabolized after initial equilibration with a half-life of approximately 3.5 days; a half-life characteristic of GpSA. No immune elimination of Fab fragments and H chains was observed. L chains obtained from purified anti-GpSA which had been injected into guinea pigs or incubated *in vitro* with GpSA prior to the injection were rapidly eliminated, similar to non-specific L chains.

DISCUSSION

The present data demonstrate that certain subunits of γ G-antibodies react *in vivo* and form complexes with circulating protein antigens. These complexes usually persist in the circulation and are eliminated with a half-life characteristic of the antigen. The *in vivo* fate of complexes formed with subunits differs from the fate of complexes formed with γ G. Although certain soluble complexes formed with γ G-antibodies also remain in the circulation for a prolonged period of time, they are eliminated faster than either antigen or antibody injected separately (2). The *in vivo* reaction of γ G-subunits with specific antigen and the persistence of the resulting complexes in the circulation appears to be an example of a protein-protein interaction resulting in a complex whose rate of elimination is governed by only one of the reactants.

The Fc fragment, which is not a part of the antibody-combining site (3), appears to be indirectly involved in the immune elimination of antigen-antibody complexes. Complexes formed by Fab and Fd fragments, which both lack the Fc fragment, remained in the circulation with the antigen. Most of the complexes formed by F (ab')₂ fragments, which also lack the Fc fragment, but still precipitate with antigen, were less effectively eliminated than complexes formed by intact γ G. Furthermore, complexes formed by F (ab')₂ fragments in slight antigen excess remained in the circulation with the antigen and were eliminated at the same rate as the antigen. It has been shown that the structure responsible for the complement-fixing property of γ G is localized mainly in the Fc fragment (22, 23). A rapid immune elimination of bacteria and erythrocytes depends, in part, on both antibody and complement (24, 25). Complement may also play a role in the immune elimination of antigen-antibody complexes formed by protein antigens. Fab fragments which no longer fix complement did not cause an immune elimination of the antigen, whereas F (ab')₂ fragments which retain a small ability to fix complement (23), eliminated the antigen less effectively than intact γ G. However, isolated specific H chains which contain the Fc fragment did not cause an immune elimination of the antigen. The H chains might

have become altered during the isolation procedure, resulting in loss of the phagocytosis promoting action related to the Fc portion of the H chain. Physicochemical changes of the antibodies occurring during the complex formation such as the formation of insoluble complexes also have to be considered as factors which enhance phagocytosis of antigen-antibody complexes.

The elimination from the circulation of γ G-subunits in presence and absence of specific antigen offers a system to study the ability of γ G-subunits to bind to antigen *in vivo*. In the absence of antigen, all γ G-subunits lacking the Fc portion of the H chain are rapidly eliminated from the circulation and catabolized (11, 12). In the presence of excess antigen, specific Fab and F (ab')₂ fragments, both known to contain one and two antibody-combining sites respectively (3, 4), remained in the circulation. Similarly, Fd fragments, either as Fd dimers or in combination with a nonspecific L chain in Fab hybrids remained in the circulation with the antigen. Although isolated H chains remained in the circulation of rabbits in the absence of antigen, specific H chains formed complexes with the antigen. After equilibration, more H chains which formed complexes remained in the intravascular compartment than H chains injected alone and when the characteristic immune elimination of the antigen occurred, the specific H chains were eliminated rapidly with the antigen. In guinea pigs nonspecific H chains were rapidly eliminated from the circulation, whereas, specific H chains remained in the circulation with the antigen. In contrast to H chains, specific L chains did not appear to bind to the antigen *in vivo*. Either isolated specific L chains or specific L chains present in Fab hybrids containing a nonspecific Fd fragment were eliminated rapidly in both presence and absence of antigen. The present findings on the ability of γ G-subunits to bind to an antigen *in vivo* are in agreement with those reported by investigators employing an *in vitro* system with a relatively high over-all recovery. These authors found that the ability to bind to an antigen remained with both the H chain and the Fd portion of the H chain, whereas the L chain did not bind significantly to antigen (5-8, 26). However, indirect evidence of binding of L chains to antigen (9, 10) as well as contribution of the L chains to the antibody-combining site have been reported (27, 28). In the present experiments, a possible binding of specific L chains to the antigen should have resulted, at least, in a temporary delay in its elimination from the circulation. However, no such delay was observed. Although these experiments do not exclude a contribution of the L chain to the antibody-combining site, they suggest that a specific contribution, if any, is small.

Although γ G-subunits interact with antigens *in vivo*, the present and previous data (11) on the *in vivo* behavior of γ G-subunits suggest that their use as prophylactic and therapeutic agents is limited. A prophylactic effect of Fab and F (ab')₂ fragments can only be brief since they are rapidly eliminated and catabolized in a host in the absence of antigen. A therapeutic effect can be expected if their function is to inactivate toxic sites of antigens, such as diphtheria and

tetanus toxins. However, if the therapeutic effect depends on the removal from the circulation of antigens, Fab fragments are ineffective and F (ab')₂ fragments appear to be only partially effective in antibody excess.

Nonspecific H chains obtained from rabbit γ G persisted in the circulation of rabbits but were rapidly eliminated from the circulation of guinea pigs. The rapid elimination of heterologous nonspecific H chains in guinea pigs might be the result of the ability of guinea pigs to excrete larger amounts of γ G-fragments in the urine (11) as well as an apparent unique catabolism of γ G in this species (29).

The rates of disappearance of both H and L chains from the circulation could be affected by the presence of contaminations. H chain preparations are difficult to obtain free of L chains which could recombine with H chains forming either intact γ G-globulin or half-molecules. The maximum contamination of the H chain preparations used in the present study was no greater than 10 to 15% and probably less. A 40% contamination, however, would be necessary to explain the persistence of H chains in the circulation of rabbits. The L chain preparations did not contain significant amounts of H chains, since following injection more than 95% of the I⁸¹ activity of the L chain preparations was rapidly eliminated from the circulation.

SUMMARY

The *in vivo* formation and subsequent fate of complexes formed between specific rabbit γ G-antibody subunits and circulating protein antigens was studied in rabbits and guinea pigs. Subunits obtained from purified antibodies were injected immediately after an injection of antigen, and the elimination from the circulation of either I^{*}-labeled γ G-subunits or labeled antigen determined.

In the absence of antigen, all γ G-subunits which lack the Fc fragment were rapidly eliminated. In the presence of excess antigen, F (ab')₂, Fab and Fd fragments reacted with antigen and remained in the circulation as complexes which were eliminated at the same rate as the antigen. Fab hybrids containing a specific Fd fragment and a nonspecific L chain similarly reacted with antigen and remained in the circulation complexed to antigen. In contrast, L chains and Fab hybrids containing a specific L chain and a nonspecific Fd fragment did not react with antigen *in vivo* and were rapidly eliminated in both presence and absence of antigen. H chains remained in the circulation of rabbits in the absence of antigen, however, in the presence of antigen, more H chains which formed complexes with antigen remained in the intravascular space and were rapidly eliminated when the immune elimination of the antigen by the host occurred. Nonspecific H chains were rapidly eliminated from the circulation of guinea pigs, whereas specific H chains remained in the circulation with the antigen.

F (ab')₂ fragments formed complexes with antigen near antibody equivalence and in antibody excess which were rapidly eliminated, however, less effectively

than complexes formed near antibody equivalence with intact γ G. Complexes formed in antibody excess between Fab fragments and H chains remained in the circulation at all concentrations studied and were eliminated at the rate of antigen.

The role of the Fc fragment in the immune elimination of antigen-antibody complexes is discussed.

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