FATE OF PREFORMED IMMUNE COMPLEXES IN RABBITS AND RHESUS MONKEYS*

By MART MANNIK and WILLIAM P. AREND

(From the Division of Rheumatology, Department of Medicine, University of Washington, Seattle, Washington 98105)

The participation of antigen–antibody complexes in the pathogenesis of vasculitis and glomerulonephritis is firmly established in several experimental animal models and human diseases. The experimentally injected antigens lead to production of antibodies. Only a small percentage of the antigens in the form of immune complexes is deposited in glomeruli during immune elimination, but tissue damage ensues (1). Similarly, in human diseases, notably in systemic lupus erythematosus, antibodies, antigens, and antigen–antibody complexes have been demonstrated in circulation (2, 3) and along the glomerular basement membrane (4). Relatively little is known, however, about what happens to the immune complexes that are formed in vivo. In particular, the characteristics of immune complexes that predispose them to removal from circulation by the reticuloendothelial system (RES)[1] and the features of immune complexes that allow them to be entrapped by vascular and glomerular basement membranes are largely unknown. Ideally this problem should be investigated in animals by following the fate of native antibodies as they react with antigen in vivo. However, due to the heterogeneity of immune response, the rapid removal of complexes, and the small amounts of antibodies involved, this approach is impractical. For these reasons the fate of preformed immune complexes was investigated in rabbits and rhesus monkeys (*Macaca mulatta*).

The observations on the elimination of soluble immune complexes from rabbit circulation (5) and the quantitative studies on tissue uptake of immune complexes (6) in rabbits have been reported previously in detail. Therefore, only the major points of the rabbit studies will be discussed.

Antibodies to antigens of variable size (λ-chains, serum albumin, γG-globulin, and γM-globulin, all of human origin) were isolated from hyperimmunized

* This work was supported by Research Grant AM11476 and Training Grant AM05602, both from the National Institute of Arthritis and Metabolic Diseases. Work at the Regional Primate Research Center was in part supported by Grant RR-00166 from the National Institutes of Health.

[1] *Abbreviations used in this paper:* Ab, antibody; Ag, antigen; BSA and anti-BSA, bovine serum albumin and antibodies to bovine serum albumin, respectively; HSA and anti-HSA, human serum albumin and antibodies to human serum albumin, respectively; RES, reticuloendothelial system.

rabbits by antigen–agarose adsorbents. The isolated γG-antibodies were labeled with ^{125}I, freed from aggregated proteins, and then used to prepare soluble immune complexes in antigen excess. The soluble complexes, made with each of the four antigens at fivefold, or higher, antigen excess, had a characteristic distribution of complexes when separated according to size by sucrose density gradient ultracentrifugation. The smallest stable or limiting complexes represented predominantly $AgAb_2$ complexes in the HSA–anti-HSA system, Ag_2Ab complexes in the λ-chain–anti-λ system, Ag_2Ab and AgAb complexes in the γG-globulin–anti-γG system and $AgAb_2$ complexes in the γM-globulin–anti-γM system. In addition, larger complexes with more lattice formation were present in each system. With increased antigen excess, the amount of the larger complexes was reduced, but the smaller characteristic complexes of each system remained, even when the excess antigen was increased manyfold. The theoretically simplest complex of Ag_2Ab was not achieved in some systems. The $AgAb_2$ and other limiting complexes were ineffective in complement fixation, but the complexes with higher degrees of lattice formation were effective in fixing complement in vitro.

When the soluble immune complexes, prepared with radio-labeled antibodies, were administered intravenously to unimmunized normal rabbits, their disappearance curves from circulation were composed of three exponential components. The first exponential component represented the elimination of immune complexes possessing more lattice formation than seen in the limiting complexes, e.g., the >11S complexes in the HSA–anti-HSA system that exceeded in lattice formation the $AgAb_2$ complexes. The half-lives of the rapidly removed complexes ranged from 0.09 to 0.25 hr. Sucrose density gradient analyses were performed on sequential serum specimens. These studies showed rapid removal of the complexes larger than the limiting antigen–antibody union and persistence of the limiting complexes and free 6.6S antibodies. The second exponential component represented equilibration of the limiting complexes and free antibodies between the intravascular and extravascular compartments, and the third exponential component represented catabolism of the same materials (see Table I for examples illustrating results with the HSA–anti-HSA system).

Reduction and alkylation of the interchain disulfide bonds of γG-globulin is known to reduce the complement-fixing efficiency of these antibodies (7). Soluble immune complexes were prepared in antigen excess with the reduced and alkylated antibodies. These preparations were ineffective in complement fixation, even though complexes with lattice formation comparable to those with intact antibodies were present. Upon administration of complexes with reduced and alkylated antibodies to rabbits, the disappearance curves from circulation were composed of two exponential components. The greater than limiting complexes persisted in circulation (e.g. the 14S-22S complexes in the

HSA-anti-HSA system) along with the limiting complexes and 6.6S antibodies. In experiments with γM-globulin as an antigen the rapid initial exponential component was diminished, but still present. In this situation it was subsequently shown that the residual RES uptake was mediated by the γM-globulin as antigen in the soluble complexes (6).

The above experiments suggested that the rapid elimination of the greater than limiting complexes was mediated by complement components in circulation. To test this possibility further, rabbits were depleted of the third component of complement with cobra venom anticomplementary factor according to the methods of Cochrane and others (8). Other rabbits were depleted of early components of complement with heat-aggregated human γG-globulin. The disappearance of circulating HSA–anti-HSA complexes was not altered in these

TABLE I

Disappearance of HSA–Anti-HSA Complexes from Rabbit Circulation: Half-Lives ($t_{1/2}$) of Exponential Components and the Percentage of Antibodies in Each Component are Provided (±1 SD)

Nature of complexes administered	First (fastest) component		Second component		Third component	
	$t_{1/2}$ (*hr*)	%	$t_{1/2}$ (*hr*)	%	$t_{1/2}$ (*hr*)	%
HSA-anti-HSA—^{125}I fivefold Ag excess	0.21 ± 0.02	41.0 ± 2.0	3.59 ± 0.47	39.8 ± 2.3	40.0 ± 5.6	19.0 ± 2.2
HSA-anti-HSA—^{125}I red and alk. fivefold Ag excess	1.98 ± 0.28	43.2 ± 2.8	63.0 ± 6.9	54.2 ± 2.8		
HSA-anti-HSA—^{125}I fivefold Ag excess rabbit received cobra venom factor	0.18 ± 0.01	46.7 ± 1.6	3.96 ± 0.46	38.1 ± 1.9	53.3 ± 9.5	16.1 ± 1.8

rabbits. The disappearance curves were still composed of three exponential components, and the proportion of antibodies eliminated with each half-life was unaltered in comparison to normal rabbits.

Subsequently experiments were conducted to quantify the uptake of immune complexes by various tissues during their rapid removal from circulation (6). Forty min was chosen as a study period because this encompassed at least three half-lives of the rapidly removed complexes. Soluble immune complexes with ^{125}I-labeled antibodies were prepared in antigen excess and administered intravenously. Five min before death each animal received ^{131}I-labeled reduced and alkylated rabbit γG-globulin or similarly treated human γG-globulin as a marker for blood volume. Thus the blood volume of each organ, the amount of complexes present in that blood, and the precise uptake of immune complexes by various organs were calculated. Upon administration of complexes with intact antibodies a large proportion of the radioactivity was found in the liver.

These counts corresponded closely to the amount of greater than limiting complexes removed from the circulation. Upon administration of similar immune complexes prepared with reduced and alkylated antibodies, only a small proportion of antibodies in the complexes was taken up by the liver (see Table II for illustrative experiments with HSA–anti-HSA systems). However, when complement components were effectively depleted with cobra venom anticomplementary factor or with aggregated γG-globulin, the hepatic RES uptake of immune complexes, prepared with intact antibodies, remained unaltered (see Table II).

During the rapid removal of immune complexes from circulation by the RES, only a very small proportion of antibodies localized in the kidneys, i.e.,

TABLE II

Tissue Distribution of Soluble HSA–Anti-HSA—^{125}I Complexes (Fivefold Antigen Excess) in Rabbits; Values are Averages of Two Experiments

Alteration of antibodies	Treatment for complement depletion	% of injected dose found in	
		Liver	Kidneys
	A: 40 min after injection		
None	None	39.3	0.15
Reduction and alkylation	None	1.3	0.21
None	Cobra venom factor	40.0	0.25
None	Aggregated γG-globulin	43.1	0.25
	B: 8–9 days after injection (during immune elimination)		
None	None	0.14*	0.03*
Reduction and alkylation	None	0.29	0.09

* Single experiment.

less than 0.5% (see Table II). Furthermore, less than 1.0% of the complexes localized in other organs. The radioactivity found in the kidneys did not represent primarily excretion of free iodine, because the isotope in homogenized renal tissue was nondialyzable. To examine the possibility of further accumulation of complexes in renal tissue, some rabbits were followed for longer periods of time after receiving the HSA–anti-HSA complexes. Rabbits were sacrificed at the time of immune elimination of the administered complexes. Further accumulation of the HSA–anti-HSA complexes was not observed in kidneys with the complexes prepared either with intact or with reduced and alkylated antibodies (see Table II for examples). Furthermore, the radioactivity that had accumulated in the liver during the rapid removal of complexes was no longer present, undoubtedly due to degradation of the phagocytized complexes and release of the radioactive iodine.

These experiments in rabbits indicated that with γG-antibodies primarily the degree of lattice formation of soluble antigen–antibody complexes dictated their rapid removal from circulation by the RES. For rapid removal of complexes the lattice formation had to exceed $AgAb_2$ complexes. The $AgAb_2$, as well as Ag_2Ab and AgAb complexes, persisted for a long time in circulation. Even though the immune complexes with more lattice formation than $AgAb_2$ fixed complement effectively, their uptake by the RES did not depend on circulating complement components, in that depletion of these components did not alter the RES uptake of complexes. Other investigators (reviewed by Huber and Fudenberg in reference 9) have delineated with in vitro experiments a receptor for γG-globulin on the surface of monocytes that does not require mediation by complement components. The rapid uptake of immune complexes by the fixed-tissue macrophages of the liver may well represent the in vivo counterpart of this phenomenon.

In order to examine the fate of immune complexes in a species closer to man, experiments were carried out in rhesus monkeys. These studies were undertaken to examine the possible role of immune adherence to red blood cells in the handling of soluble antigen–antibody complexes. The immune adherence receptor is present on primate erythrocytes, but not on the red blood cells of lower species such as the rabbit (10).

Materials and Methods

Preparation of Antigens, Antibodies, and Immune Complexes.—Bovine serum albumin (BSA) (crystallized BSA from Mann Research Laboratories, Inc., New York) was freed from aggregates by gel filtration over a column of Sephadex G-200 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) (5 cm^2 × 95 cm volume; equilibrated with 0.2 M sodium borate, 0.15 M NaCl, pH 8.0). Four rhesus monkeys (*Macaca mulatta*) were hyperimmunized with BSA in complete Freund's adjuvant. Serial serum samples were obtained over several months and the serum specimens were pooled. The specific antibodies were isolated with a BSA–agarose immunoadsorbent by techniques described in earlier studies (5). The isolated antibodies were labeled with ^{125}I, and for some studies BSA was labeled with ^{131}I. These trace-labeled proteins were freed of unbound iodine and aggregated molecules. The concentrations of anti-BSA–^{125}I and BSA were determined by absorbance at 280 mμ utilizing extinction coefficients (1% solutions, 1-cm light path) of 13.7 and 6.6, respectively, for the two proteins. Aliquots of the labeled antibodies were reduced and alkylated before the final gel filtration.

Precipitin curves were constructed with monomeric BSA and anti-BSA–^{125}I, and the point of equivalence was determined. Soluble immune complexes were prepared at the desired degree of antigen excess by adding the solution of antibodies to the solution of antigen under constant agitation. The size and heterogeneity of the soluble complexes were determined by linear sucrose density gradients, and complement-fixing ability was assayed, all by previously detailed methods (5).

Elimination of Immune Complexes from Circulation.—The soluble immune complexes, or other test materials, were injected into the sural vein of unimmunized rhesus monkeys (3–4 kg), which were sedated lightly with Valium and strapped to a contoured board. Blood samples (2–3 ml) were obtained from other veins and anticoagulated with heparin or allowed to clot. Initial specimens were obtained at frequent intervals, but subsequently two to three

bleedings were obtained per day. After the initial hour, monkeys were housed in cages and strapped only for bleeding purposes. The amount of antibody injected in complexes ranged from 5 to 10 mg per monkey, containing 5–10 μCi of radioactivity. The plasma or serum was harvested and stored at 4°C until precise aliquots were pipetted for counting. In some experiments accurate aliquots of blood were obtained along with serum specimens for counting. The amount of radioactivity in sequential specimens was determined. The per cent of remaining isotope was calculated and plotted on semilogarithmic paper against time. The disappearance curves were analyzed graphically and by a computer program for the number of exponential components and their half-lives as previously described (5).

Tissue Localization of Immune Complexes.—In these experiments the BSA-anti-BSA—^{125}I complexes at fivefold antigen excess were administered intravenously. Thirty min after injection of complexes, reduced and alkylated human γG-globulin–^{131}I was administered as a marker for blood volume. Five min later the monkeys were sacrificed. In this manner the

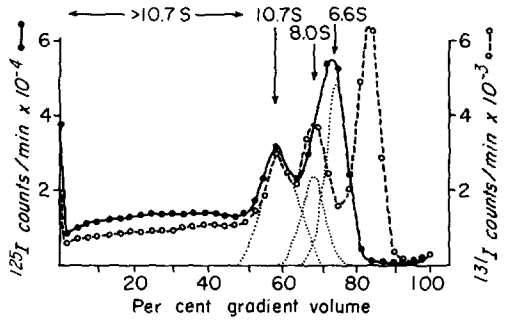

FIG. 1. Density gradient ultracentrifugation pattern of rhesus BSA-^{131}I—anti-BSA-^{125}I complexes at fivefold antigen excess. A gradient of 10–40% sucrose and SW41Ti rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) were used; top of the gradient is represented by 100% of the gradient volume. The dotted (....) lines indicate the distribution of labeled antibodies in 6.6S, 8.0S, and 10.7S components.

uptake of immune complexes by various tissues was determined as previously described (6). The results were expressed as the percentage of injected antibodies taken up by each organ.

RESULTS

Rhesus antibodies to BSA were isolated and labeled with ^{125}I and immune complexes were prepared at fivefold antigen excess with BSA–^{131}I. These complexes were characterized by density gradient ultracentrifugation (see Fig. 1). Free antigen was present, but two additional discrete peaks of antigen were observed with sedimentation coefficients of 8.0S and 10.7S. A broad shoulder of faster sedimenting complexes was also present. Free antibodies were present but the peak was asymmetrical with a skew towards the bottom of the centrifuge tube. With a Dupont 310 curve resolver (E. I. DuPont de Nemours & Co., Wilmington, Del.) this peak was separable into free 6.6S antibodies and 8.0S complexes. A clearly identifiable 10.7S peak was present, and >10.7S complexes

were observed. The molecular weight of the 8.0S and 10.7S peaks were calculated according to the method of Martin and Ames (11) as 194,000 and 299,000, respectively. In the 8.0S peak the molar ratio of antibody to antigen could not be calculated accurately because of the close proximity to free 6.6S antibodies, but the estimated values tended to be above unity. In the 10.7S peak the molar ratio of antibody to antigen was 1.0 in several experiments. In view of these results the 8.0S complexes were thought to be composed primarily of Ag_1Ab_1. Since the molar ratio of antibody to antigen in the 10.7S complexes was close to 1.0, these complexes were thought to be composed of Ag_2Ab_2 union, even though the estimated molecular weight was considerably less than

TABLE III

Disappearance of Isolated Antibodies, BSA, and BSA–Anti-BSA Complexes from Circulation of Rhesus Monkeys: Half-Lives ($t_{1/2}$) of Exponential Components and the Percentage of Radio-Labeled Material in Each Component are Provided (±1 SD)

Nature of injected material	First (fastest) component		Second component		Third component	
	$t_{1/2}$ (*hr*)	%	$t_{1/2}$ (*hr*)	%	$t_{1/2}$ (*hr*)	%
Anti-BSA-^{125}I	1.28 ± 0.45	12.4 ± 2.0	59.7 ± 3.1	87.0 ± 2.0		
Rhesus γG-^{131}I	1.24 ± 0.50	11.6 ± 2.4	50.3 ± 2.6	87.9 ± 2.4		
BSA-^{131}I	4.28 ± 0.70	43.2 ± 3.4	86.5 ± 11.4	52.2 ± 3.3		
BSA-anti-BSA—^{125}I fivefold Ag excess	0.16 ± 0.06	18.3 ± 3.7	2.25 ± 0.45	39.0 ± 3.7	75.5 ± 11.6	42.9 ± 2.6
BSA-anti-BSA—^{125}I fivefold Ag excess	0.22 ± 0.06	37.0 ± 7.8	1.91 ± 2.02	12.3 ± 8.4	43.1 ± 5.3	51.7 ± 3.7
BSA-anti-BSA—^{125}I red. and alk. fivefold Ag excess	4.45 ± 0.87	33.4 ± 3.4	82.0 ± 9.8	65.2 ± 3.4		

expected. The >10.7S complexes fixed complement effectively, in that 0.38 μg of antibody was required to fix 50% of the guinea pig complement. On the other hand the Ag_2Ab_2 complexes in 10.7S peak required more than 36 μg of antibody to fix the same amount of complement.

The half-lives of the isolated anti-BSA-^{125}I and normal rhesus γG-globulin-^{131}I were found to be comparable (see Table III). Of interest was that 87% of these proteins remained in the intravascular space. The half-life of BSA in a rhesus monkey was 86.5 hr, and this protein distributed nearly equally between the intravascular and extravascular compartments.

The disappearance curves of the BSA–anti-BSA—^{125}I complexes were composed of three exponential components (see Fig. 2 and Table III). The first component had a half-life of 0.16 and 0.22 hr in two separate experiments. Density gradient experiments of sequential serum specimens during the elimina-

tion of complexes disclosed that the >10.7S complexes were quickly removed, whereas the 10.7S and 8.0S complexes and 6.6S antibodies persisted (see Fig. 3). When similar complexes were prepared with reduced and alkylated antibodies, their removal from circulation had only two exponential components. Density gradient analyses disclosed the persistance of the >10.7S complexes along with the other components (see Figs. 2 and 3 and Table III). Of interest was that in some experiments the distribution as well as half-life of the complexes that

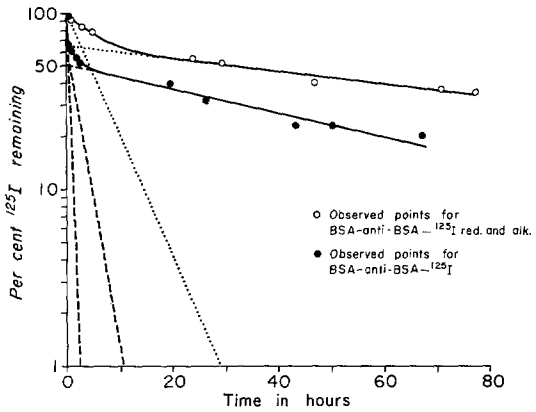

FIG. 2. Disappearance of BSA-anti-BSA—^{125}I complexes with intact and reduced and alkylated antibodies in rhesus monkeys. The solid circles (●) indicate the experimentally observed points for the disappearance of BSA-anti-BSA—^{125}I complexes. The solid line (——) indicates the curve fitted to these points by computer. The dashed (— —) lines indicate the three exponential components that compose this curve. The open circles (○) indicate the experimentally observed points for the disappearance of BSA-anti-BSA—^{125}I complexes with reduced and alkylated antibodies. The solid line indicates the curve fitted to these points by computer. The dotted (....) lines indicate the two exponential components that compose this curve.

remained in circulation approximated these parameters of the antigen (BSA) rather than of the free antibodies.

Quantitative tissue uptake of complexes was determined with antigen–antibody complexes prepared with intact antibodies as well as with complexes prepared with reduced and alkylated antibodies. With the former the hepatic uptake represented the removal of >10.7S complexes. Less than 1.0% of the complexes made with reduced and alkylated antibodies were taken up by the liver during 40 min (see Table IV). Less than 0.5% of antibodies were taken up by several other organs, including the kidneys. In these experiments 87–99% of the administered radioactivity was accounted for by the counts in the examined tissues and blood.

With the BSA–anti-BSA complexes at fivefold antigen excess no evidence

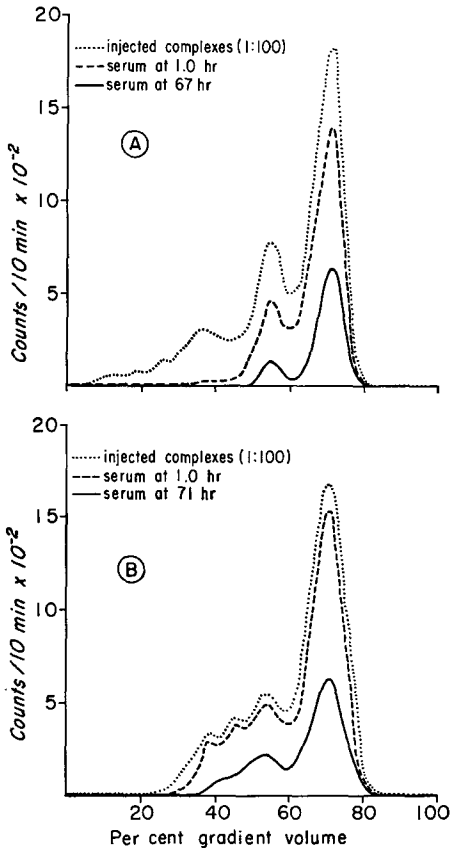

FIG. 3. Density gradient ultracentrifugation patterns of sequential serums of monkeys which had received immune complexes. The conditions of centrifugation were the same as in Fig. 1. In (A) the diluted BSA-anti-BSA—^{125}I complexes at fivefold antigen excess are compared with complexes remaining in circulation; the >10.7S complexes are removed quickly. In (B) the diluted BSA-anti-BSA—^{125}I complexes at fivefold antigen excess prepared with reduced and alkylated antibodies are compared with complexes remaining in circulation; the >10.7S complexes are not selectively and quickly removed from circulation.

TABLE IV

Tissue Distribution of Soluble BSA-Anti-BSA—^{125}I Complexes (Fivefold Antigen Excess) in Rhesus Monkeys 40 min after Injection

Alteration of antibodies	% of injected dose found in			
	Liver	Kidneys	Spleen	Lungs
None	24.0	0.05	0.3	0.5
None	16.4	0.02	0.2	0.2
Reduction and alkylation	0.6	0.06	0	0.2

for immune adherence to red cells was found in vivo or in vitro. If immune adherence occurred to red cells upon intravenous injection of complexes, then the removal of complexes from serum specimens should exceed the removal of complexes from whole blood. However, the per cent of administered complexes remaining during the first 8 hr were identical for blood and serum studies in two experiments. Evidence for immune adherence to erythrocytes was sought by in vitro experiments. When preformed BSA–anti-BSA—^{125}I complexes were incubated with washed erythrocytes (human or rhesus) and with either fresh or heated serum (human or monkey), the immune complexes did not bind to erythrocytes in the presence of complement. Furthermore, negative results were obtained even when the immune complexes at fivefold antigen excess were allowed to form in the presence of red cells and complement. The reasons are not apparent for absence of immune adherence of the BSA–anti-BSA complexes either to human or rhesus red cells in the presence of appropriate complement components.

DISCUSSION

The results of studies in rabbits and monkeys clearly show that the hepatic RES quickly removes circulating immune complexes that contain more than two γG-antibodies, that is, complexes with more lattice formation than seen in Ag_1Ab_2 or Ag_2Ab_2. Immune complexes containing the same class of antibodies, but consisting of Ag_2Ab_2, Ag_1Ab_2, Ag_1Ab_1, or Ag_2Ab_1 circulated for many hours. The half-life of these complexes tended to approach the half-life of the antigen alone in several systems.

The participation of the antigen, incorporated into the soluble complexes, in the rapid removal of immune complexes by the RES was well demonstrated by experiments with human γM-globulin as an antigen (6). The aggregates of this protein were quickly removed from the circulation of rabbits by the hepatic RES. It was also demonstrated that the rapid removal of complexes made with this antigen and reduced and alkylated rabbit antibodies was mediated by the human γM-globulin as antigen in the complexes. In contrast to the situation with rabbit γG-antibodies, the RES uptake mediated by human γM-globulin was dependent upon the third component of the complement system. Even more striking was the rapid removal of all soluble immune complexes made with the 7S subunits of human γM-globulin as an antigen. The latter alone was also quickly taken up by the RES of rabbits. However, in the antigen–antibody systems where the antigen in the complex was not reactive with fixed-tissue macrophages, the mechanisms of catabolism of the limiting complexes remain unknown. For that matter, the sites and mechanisms of catabolism of free immunoglobulins are largely undefined. Thus the mechanisms of disposal and catabolism of complexes not rapidly removed by the RES remain to be elucidated.

The studies on disappearance of circulating immune complexes in rhesus monkeys confirm the conclusions of previously performed similar investigations in rabbits. The failure to find any evidence for adherence of soluble BSA–anti-BSA complexes to rhesus or human erythrocytes is of interest. However, the in vivo significance of this receptor has not been defined.

In experimental animals and in human diseases, as reviewed elsewhere in this symposium, virus–antibody complexes have been observed to persist in circulation. The role of γG-antibodies, and the density of such antibodies on the virus particle, in relation to the removal of these complexes by the RES would be of interest.

The presented data suggest that immune complexes possessing little lattice formation are not rapidly removed by the RES, irrespective of their size. The bulk of immune complexes demonstrable in the sera of patients with immune complex diseases might have relatively simple lattice formation in that complexes with higher degrees of lattice formation would be in circulation only transiently. Alternatively, the possibility exists that these circulating complexes primarily contain antibodies which interact poorly with the fixed-tissue macrophages of the RES. In vitro studies have demonstrated that the γG-globulin receptors on monocytes are limited to subclasses 1 and 3 of this class of immunoglobulins (9). The precise characteristics of circulating complexes found in human diseases, including molecular ratio and antibody subclass composition, have not been determined. Also the possible persistence of complexes with greater than Ag_2Ab_2 lattice formation in tissue spaces with limited monocyte availability, e.g. the synovial cavity, warrants further investigation.

The immune complexes made with reduced and alkylated γG-antibodies and containing more lattice formation than Ag_2Ab_2 were not quickly removed from circulation by the RES. These larger complexes remained in circulation along with the complexes with lesser degrees of lattice formation. The above mentioned chemical alteration of γG-antibodies is known to render them ineffective in complement fixation (7). Thus the possibility was considered that complement components mediate the rapid removal of immune complexes by the fixed-tissue macrophages. However, when rabbits were depleted of complement, the RES uptake of complexes made with intact antibodies was not altered. Therefore, the circulating complement components do not play a role in removal of circulating immune complexes. It is known that macrophages from many tissues are capable of synthesizing complement components. In the liver, however, complement appears to be synthesized by the parenchymal cells and not by the Kupffer cells (12). The participation of interstitial or cell-bound complement components in the RES uptake of immune complexes was not excluded by our experiments. The fate of immune complexes formed with other classes of antibodies has not been defined, and the mechanisms for clearance of these complexes have not been elucidated.

Quantitative studies on the tissue localization of immune complexes showed that only a very small proportion (less than 1.0%) of the circulating immune complexes localized in the kidneys of rabbits and rhesus monkeys. This was true in short-term experiments as well as in experiments when complexes circulated for several days. The degree of lattice formation of immune complexes that are entrapped by the vascular or glomerular basement membranes has not been defined. Apparently the physical size of immune complexes, as well as of inert substances, has to exceed a minimum before they can effectively be entrapped by these basement membranes during conditions of increased vascular permeability (13).

SUMMARY

Preformed soluble immune complexes injected into rabbits or rhesus monkeys showed similar characteristics of disappearance from circulation. Complexes made with intact γG-antibodies and exceeding the Ag_2Ab_2 lattice formation were rapidly removed by the hepatic RES. These complexes fixed complement effectively in vitro. Their hepatic uptake was not dependent upon circulating complement components, since their accumulation in the liver was unchanged in complement depleted rabbits. Similar antigen–antibody complexes made with reduced and alkylated γG-antibodies fixed complement ineffectively in vitro. These complexes possessed different disappearance characteristics and were not rapidly taken up by the liver, regardless of their degree of lattice formation. Both in vitro and in vivo studies failed to suggest any role for the immune adherence receptor on primate erythrocytes in the handling of circulating soluble immune complexes composed of BSA and γG-antibodies to this antigen.

We thank Mr. R. Aeschliman and Miss Anne Grant for their technical assistance.

BIBLIOGRAPHY

1. Wilson, C. B., and F. J. Dixon. 1970. Antigen quantitation in experimental immune complex glomerulonephritis. I. Acute serum sickness. *J. Immunol.* **105:** 279.
2. Tan, E. M., P. H. Schur, R. I. Carr, and H. G. Kunkel. 1966. Deoxyribonucleic acid (DNA) and antibody to DNA in the serum of patients with systemic lupus erythematosus. *J. Clin. Invest.* **45:**1732.
3. Agnello, V., R. J. Winchester, and H. G. Kunkel. 1970. Precipitin reactions of the Clq component of complement with aggregated γ-globulin and immune complexes in gel diffusion. *Immunology.* **19:**909.
4. Koffler, D., P. H. Schur, and H. G. Kunkel. 1967. Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. Exp. Med.* **126:**607.
5. Mannik, M., W. P. Arend, A. P. Hall, and B. C. Gilliland. 1971. Studies on antigen–antibody complexes. I. Elimination of soluble complexes from rabbit circulation. *J. Exp. Med.* **133:**713.

6. Arend, W. P., and M. Mannik. 1971. Studies on antigen-antibody complexes. II. Quantification of tissue uptake of soluble complexes in normal and complement-depleted rabbits. *J. Immunol.* In press.
7. Schur, P. H., and G. D. Christian. 1964. The role of disulfide bonds in the complement-fixing and precipitating properties of 7S rabbit and sheep antibodies. *J. Exp. Med.* **120:**531.
8. Cochrane, C. G., H. J. Müller-Eberhard, and B. S. Aikin. 1970. Depletion of plasma complement *in vivo* by a protein of cobra venom: its effect on various immunologic reactions. *J. Immunol.* **105:**55.
9. Huber, H., and H. H. Fudenberg. 1970. The interaction of monocytes and macrophages with immunglobulins and complement. *Ser. Haematol.* **3:**160.
10. Nelson, D. S. 1963. Immune adherence. *Advan. Immunol.* **3:**131.
11. Martin, R. G., and B. N. Ames. 1961. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. *J. Biol. Chem.* **236:** 1372.
12. Stecher, V. J. 1970. Synthesis of proteins by mononuclear phagocytes. *In* Mononuclear Phagocytes. R. van Furth, editor. F. A. Davis Company, Philadelphia. 133.
13. Cochrane, C. G., and D. Hawkins. 1968. Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localize in blood vessels. *J. Exp. Med.* **127:**137.