# Interaction of Immunoglobulins with Liposomes

GERALD WEISSMANN, ANN BRAND, and EDWARD C. FRANKLIN

*From the Department of Medicine, Rheumatic Diseases Study Group, and The Irvington House Institute, New York University School of Medicine, New York 10016*

ABSTRACT Liposomes were used as model targets to test the effect of immunoglobulins on biomembranes. Heat-aggregated immunoglobulins (Ig) exceeded native immunoglobulins in their capacity to release anions and glucose from model liposomes (either lecithin-dicetylphosphate-cholesterol or lecithin-stearylamine-cholesterol in molar ratios of 7:2:1). This interaction was not dependent upon the presence of cholesterol in the membrane. Mild heat-aggregation (10 min at 61.5°C) increased the membrane-perturbing activity of certain Ig. Activity varied among classes and subclasses: $IgG_1$ > pooled IgG > $IgG_4$ > $IgA_1$ > $IgG_3$. $IgG_2$, $IgA_2$ and IgM were inert. Fc fragments of IgG were as active as $IgG_1$, whereas Fab fragments were inactive. Prolonging aggregation to 60 min destroyed the activity of Ig. Membrane-activity could not be induced in non-Ig molecules (such as bovine serum albumin) by 10 or 60 min heat-aggregation. Density gradient centrifugation of $IgG_1$ molecules indicated that membrane perturbing activity was associated with 15–20-s aggregates. Sepharose 4B chromatography demonstrated preferential interaction between cationic membranes and aggregated Ig, whereas anionic membranes interacted nonselectively with both native and aggregated Ig via salt-like interactions. One explanation for these data is that heat aggregation induces a conformational change in the Fc regions of certain Ig permitting them to interact with liposomes, presumably by enhancing their hydrophobic associations with membrane phospholipids.

## INTRODUCTION

Mechanisms of interaction between immunoglobulins (Ig)[1] and biomembranes are pertinent to at least two areas of immunology. The first is the triggering of B cells by the interaction of antigen with surface immunoglobulins of lymphocyte membranes. Ig of various classes and subclasses have been localized on the surface of B lymphocytes (1, 2), are synthesized by these cells, can be removed by trypsin, and are not replaced by exposing the cells to fresh serum (1). The second is the specific interaction of immunoglobulins with the surfaces of several types of cells, which, in the case of phagocytes, are stimulated by this encounter to ingest immune complexes or coated particles. This second type of interaction has been postulated to occur between externally derived Ig and discrete "receptor" sites on cell membranes; such sites are insensitive to treatment with trypsin (3–5). Receptors for immunoglobulins are found on monocytes (6–8), macrophages (9, 10), and neutrophils (11, 12) as well as on B lymphocytes (13–15) and resemble receptors for cytophilic antibody (3, 16, 17). They react with antigen-antibody complexes, bind antibody independently of divalent cations, and appear to bind the Fc region of the Ig molecule (17, 18). On lymphocytes, these receptors appear to differ from surface Ig (13) and complement binding sites (3, 19). Nevertheless, it is by no means clear that some of these interactions are not due to a nonspecific affinity for lipid surfaces.

Ig are known to undergo a conformational change both during the formation of antigen-antibody complexes and upon heat aggregation. The combination of antigen with antibody has been visualized in the electron microscope as a "clicking open" of the Ig molecule (20). The configurational change that occurs in the Ig molecule after both heat aggregation and Ag–Ab complex formation renders the protein biologically active with respect to complement fixation and the reactions of cutaneous hypersensitivity (21, 22). Recently, heat aggregation of Ig has been shown to result in an Ig molecule that is

*Received for publication 20 July 1973 and in revised form 10 September 1973.*

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; Ig, immunoglobulin(s); PBS, phosphate-buffered saline.

more "membrane-active" than its native species (12, 23, 24), but the nature of this activity is as yet unclear.

Consequently, we have studied the interactions of Ig with liposomes, aqueous dispersions of phospholipids in the smectic mesophase (25). At appropriate temperature and concentration, phospholipids form concentric lipid bilayers capable of ion discrimination. Operationally, the model membranes are separated by aqueous compartments which can trap various small molecules or ions, by virtue of the repulsive forces imposed on the membranes by the incorporation of either long-chain anions or long-chain cations. These model membranes have been used to study the action of agents such as steroids, lytic proteins, and polyene antibiotics (26–28), which react with liposomes much as with natural membranes. More recently liposomes have been used to demonstrate the interaction of various complement components with membranes (29, 30).

The experiments described below were designed to test the hypothesis that Ig, conformationally altered by heat aggregation, would display enhanced capacity to interact with, and perturb, the membranes of liposomes as measured by passive diffusion of small molecules across the bilayers.

## METHODS

*Immunoglobulins.* Normal IgG was prepared from Cohn fraction II by chromatography on DEAE cellulose (31). Myeloma proteins and macroglobulins were isolated either by starch zone electrophoresis, or by starch zone electrophoresis followed by chromatography on Sephadex G-200 (32). Purity of all preparations was determined by immunoelectrophoresis (33). Classes and subclasses were determined with specific antisera (34) as well as by chemical typing (35). Fab and Fc fragments were prepared with papain and purified as described (36). Heavy and light chains were prepared by reduction with 0.1 M 2-mercaptoethanol, followed by alkylation with 0.2 M iodoacetamide and purified by filtration on Sephadex G-100 in 1 M acetic acid.

*Preparation of liposomes.* Liposomes were prepared by a slight modification of previously described methods (27, 28). Suspensions with a net negative charge were prepared from a mixture of either lecithin-dicetylphosphate-cholesterol in a molar ratio of 7:2:1 or lecithin-dicetylphosphate in a molar ratio of 8:2. Liposomes with a net positive charge were prepared from a mixture of lecithin-stearylamine-cholesterol in a molar ratio of 7:2:1. Lecithin was purchased from General Biochemicals, Chagrin Falls, Ohio; cholesterol from Fisher Scientific Co., Fair Lawn, N. J.; and dicetylphosphate and stearylamine from K & K Laboratories, Inc., Plainview, N. Y. The lipids were dissolved in chloroform and added to a 100-ml round bottomed flask. A rotary pump apparatus was used to evaporate the chloroform, resulting in the deposition of a uniformly thin layer of lipid on the walls of the flask. After the chloroform was completely evaporated, 6 ml of "swelling" solution (0.145 M $K_2CrO_4$, 0.29 M glucose) was added. Gentle shaking using a vortex mixer dislodged the lipid film from the walls of the flask. Next the lipid mixture (approximately 15 μmol lipid/ml) was disrupted by 8 min of sonic oscillation in a Branson Automatic Cleaner (Model HD-50, Heat Systems Co., Melville, N. Y.), resulting in a turbid suspension of swollen, hydrated liposomes which was permitted to stand for 2 h. These suspensions were layered on top of a column of Sephadex G-50 (coarse), in the case of negatively charged dispersions, or Sephadex G-75 for positively charged dispersions. Liposomes were eluted from the columns (2.5 × 22 cm) with equimolar NaCl/KCl (0.145 total molarity). This procedure was used to separate the liposomes from marker which had not become trapped within the membranes.

The extent of release of ions or glucose from liposomes was determined by mixing 1 ml samples of the suspended spherules in small dialysis bags with various concentrations of complement-free, purified Ig or myeloma proteins dissolved in phosphate-buffered saline (PBS) ($NaCl/PO_4$, Grand Island Biological Co., Grand Island, N. Y., No. 404-IX). Aggregated Ig was prepared by heating the dissolved native protein in a 61.5°C water bath for 10 min. The dialysis bags containing Ig and liposomes were placed in test tubes which contained 5.0 ml of 0.145 M NaCl/KCl and then incubated in a shaking water bath at 37°C. To determine the integrity of liposomes, release was measured of anions or glucose from bags containing the liposomes alone or liposomes + 0.2 ml of Triton X-100 (Rohm & Haas, Philadelphia, Pa.) (2% vol/vol); the detergent releases "total" sequestered ions or glucose. Leakage of marker from the bags into the saline was determined at 30, 60, and 90 min intervals. Chromate leak was measured by its absorbance at 370 nm. Glucose leak was determined by the glucose oxidase method (37). In some experiments, the specific effect of aggregated fractions was expressed as the percentage (vs. controls at 90 min) of anions or glucose released by aggregated minus that released by native proteins.

*Sucrose density gradient centrifugation.* Native and aggregated IgG were layered on top of a sucrose gradient (12.5–25–37.5% sucrose) and sedimented in a Spinco Model L Ultracentrifuge (SW-39 rotor; Beckman Instruments, Spinco Div., Palo Alto, Calif.) for 21 h at 32,000 rpm. Fractions were collected from a pinhole in the bottom of the cellulose nitrate centrifuge tubes. Sucrose was removed by dialysis for 2–3 days against several changes of 0.145 M NaCl/KCl. Protein concentration in each of the fractions was determined by the Lowry method and immunoglobulins were localized by testing the fractions with antisera to IgG by Ouchterlony analysis. The fractions were adjusted to equivalent concentrations with PBS before testing their effect on liposomes.

*Chromatography.* To determine whether IgG became associated with lipid bilayers, native or aggregated IgG after gradient separation (see above) was mixed with either anionic or cationic liposomes. The IgG-liposome mixture (final concentration = 150 μg IgG/ml of liposome) was allowed to stand for 10 min at room temperature. The mixture was then placed on a Sepharose 4B column 2.6 × 45 mm (Pharmacia Fine Chemicals, Piscataway, N. J.) with 0.145 M NaCl/KCl to separate membrane-associated protein from unbound material.

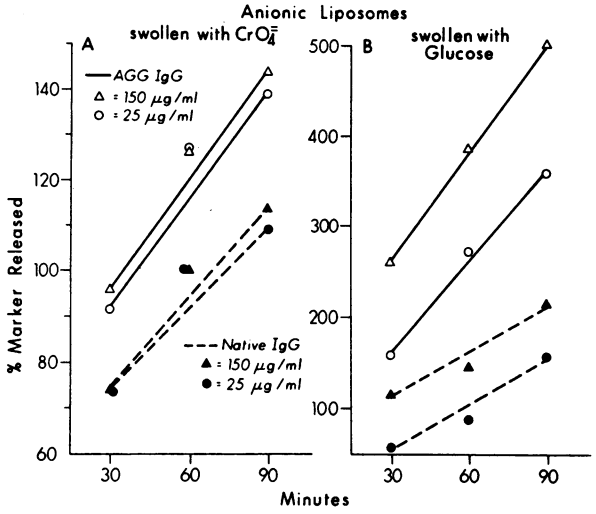


FIGURE 1 The release of (*A*) anions and (*B*) glucose from liposomes by increasing concentrations of native and aggregated (10 min) IgG. Liposomes (1.0 ml prepared with lecithin-dicetylphosphate-cholesterol in molar ratios of 7:2:1, 15 μmol lipid/ml) were mixed with indicated amounts of native or aggregated IgG in dialysis bags and incubated for 90 min at 37°C. Leak of $CrO_4^=$ from the control at 90 min = 70 μg/ml; total anion trapped = 800 μg/ml; total glucose from the control at 90 min = 2 μg/ml; total glucose trapped = 18 μg/ml. The results are expressed as the percent of anion or glucose released by the control sample (15 μmol of lipid alone) at 90 min.

## RESULTS

### The release of chromate and glucose from anionic liposomes by native and aggregated immunoglobulins.

Native and heat-aggregated IgG were added to anionic liposomes ($CrO_4^=$ or glucose as markers) and release of these markers was followed. Preliminary studies indicated that IgG at concentrations lower than 15 μg/ml and higher than 150 μg/ml [2] resulted in no detectable difference between the amount of marker released by native and aggregated IgG. When, however, Ig were prepared so that their final concentrations fell within this range, leakage of $CrO_4^=$ or glucose resulting from addition of aggregated IgG was significantly greater than that which occurred in the presence of native protein (Fig. 1).

Since aggregated Ig released glucose (a nonelectrolyte) even more vigorously than $CrO_4^=$, release of markers by Ig would seem to result from interactions between proteins with the lipid membrane rather than by the counter-ion effects of cationic Ig upon $CrO_4^=$. Indeed Kaplan (38) has demonstrated that $CrO_4^=$ efflux rates vary with the nature of the net ionic charge of liposomes and are enhanced by lysozyme. Enhanced efflux (vs. control) of both anions and glucose from anionic liposomes in the presence of native IgG is in keeping with the sensitivity of liposomes to coulombic disruption by cationic proteins. These effects, can however, be distinguished from the *specific* enhancement of anion or glucose efflux by simply subtracting the efflux in the presence of native Ig from that in the presence of aggregated proteins (Fig. 2).

By these means, different classes and subclasses of Ig were examined to determine their relative membrane perturbing ability. Aggregated $IgG_1$, $IgG_3$, and $IgG_4$ exceeded the native species in provoking release of anions or glucose, whereas $IgG_2$ had little effect. Aggregated $IgA_1$ also acted to perturb lipid membranes. In initial experiments, no enhanced release of $CrO_4^=$ was induced by aggregated, as compared with "native" $IgA_1$. However, in subsequent studies, the native $IgA_1$ was sedimented (40,000 rpm for 2 h) to remove any aggregated protein. When the supernatant fluid was used as the native sample and compared with another sample which was aggregated for 10 min at 61.5°C, it was shown that aggregation increased the membrane perturbing activity

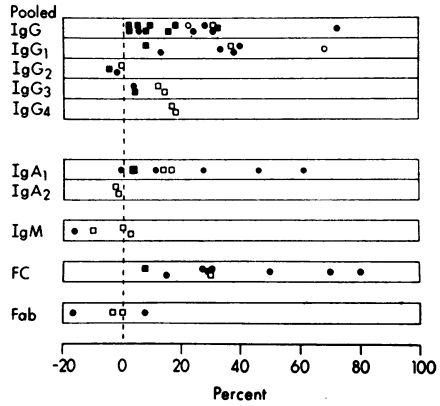


FIGURE 2 "Specific" release of anion or glucose from anionic liposomes (lecithin-dicetylphosphate-cholesterol in molar ratios of 7:2:1) by aggregated (10 min) vs. native immunoglobulins of different classes and subclasses. Liposomes incubated at 37°C with immunoglobulins (150 μg/15 μmol lipid/ml). *Specific release* (aggregated-native) is expressed as percentage increment or decrement vs. control samples (15 μmol lipid alone) which released (mean values) 70 μg/ml $CrO_4^=$ and 2 μg/ml glucose at 90 min. Thus only differences between native and aggregated samples are shown. Each horizontal bar represents a different class or subclass, and each point represents the mean of three separate determinations on an individual protein of that class or subclass. ●, glucose leak of using unsedimented native Ig; ○, glucose leak using supernates of sedimented, native Ig; ■,$CrO_4^=$ leak using unsedimented native Ig; □, $CrO_4^=$ leak using supernates of sedimented native Ig.

[2] Molar ratios of protein/lipid between 1/10,000 and 1/1,000.

of $IgA_1$. $IgA_2$ was less effective in provoking anion and glucose efflux, and IgM did not provoke release of anions or glucose from the liposomes. Preliminary sedimentation of these proteins followed by heat aggregation did not induce enhanced release by the aggregated samples. Fc fragments acted to perturb liposomes, and their activity was comparable to that of $IgG_1$, whereas Fab fragments were found to be inactive. These fragments were studied at protein concentrations equal to those of the whole protein rather than at appropriate molar ratios, because varying the protein-lipid ratio would have enhanced lipid/lipid interactions, i.e. membrane stabilization (38).

In similar experiments with IgG, cholesterol was omitted from the anionic lipid mixture, resulting in lecithin-dicetylphosphate liposomes with molar lipid ratios of 8:2. Such liposomes responded with enhanced release of $CrO_4^=$ or glucose on the addition of aggregated as compared with native pooled IgG to the same degree as did liposomes which contained sterol.

Cationic liposomes, prepared with cholesterol, responded to aggregated, pooled IgG in the same fashion as did anionic liposomes (e.g. 360% of control glucose release in the presence of 25 μg/ml of aggregated vs. 140% of control in the presence of 25 μg/ml of native protein).

## Relationship between extent of aggregation and marker release

It remained possible that the enhanced release of anions or glucose in response to aggregated Ig was due to nonspecific denaturation of the molecule, rather than to a more specific conformational change assumed by the protein after gentle heating. To test this possibility, the period of Ig aggregation was extended to 60 min. This treatment markedly diminished the activity of Ig which had been heated for only 10 min (Fig. 3).

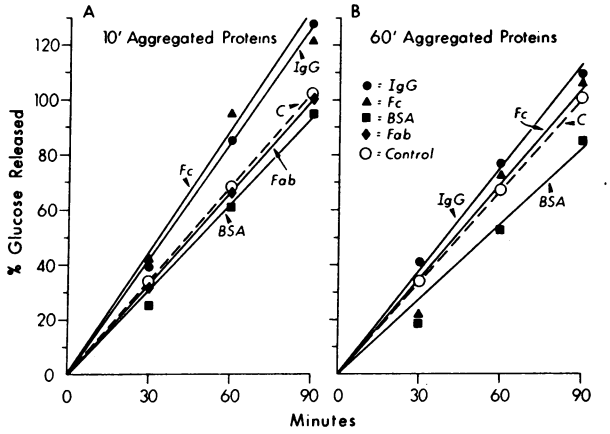


FIGURE 3 The effect of the duration of aggregation (10 vs. 60 min) on the amount of glucose released from anionic liposomes. The anionic liposomes were incubated with various immunoglobulins or BSA (final concentration = 90 μg/ml protein/15 μmol lipid per ml) which had been aggregated for either 10 or 60 min. Glucose efflux measured at 30, 60, and 90 min and expressed as the percentage of glucose released by the control sample in the absence of protein after 90 min (2 μg/ml). Each point represents the mean of three experiments. (*A*) Results after 10 min aggregation; (*B*) results after 60 min aggregation.

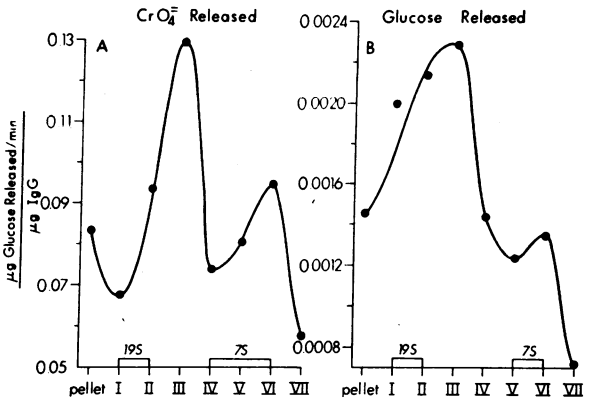


FIGURE 4 The release of anions and glucose from anionic liposomes by fractionated IgG. Liposomes were prepared with lecithin-dicetylphosphate-cholesterol in a molar ratio of 7:2:1. IgG was dissolved at high concentrations (20–35 mg/ml), aggregated at 61.5°C for 10 min, layered on a sucrose gradient (12.5–37.5% sucrose), and sedimented at 32,000 rpm for 21 h (SW 39 rotor). Fractions (I–VII) adjusted to the same protein concentration were incubated for 90 min at 37°C with liposomes (30 μg protein/15 μmol lipid per ml). The results are expressed in micrograms of glucose leaked per minute per microgram of IgG. (*A*) Anion ($CrO_4^=$) leak from liposomes swollen in 0.145 M $K_2CrO_4$. (*B*) Glucose leak from liposomes swollen in 0.29 M glucose plus 0.145 M NaCl. 7S and 19S indicate the location of molecules of that size.

## The effects of non-Ig protein on anionic liposomes

Bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) was chosen to study the capacity of non-Ig proteins to release glucose trapped within negatively charged lipid bilayers. A 10 min period of aggregation at 66.5°C was chosen because such aggregates most nearly resemble the size of IgG which had been aggregated for 10 min at 61.5°C (39). This period of aggregation did not render the BSA membrane active. Lengthening the time of heating to sixty minutes (Fig. 3) or even to 24 h (data not shown) did not result in increased activity. Although all proteins become less membrane-active after denaturation for 60 min, these experiments demonstrate that membrane perturbation is not a property of denatured proteins in general.

## Release of anions or glucose by various sized aggregates of IgG

Since even so-called native proteins contain varying amounts of denatured materials, sucrose density centri-

fugation was used to determine the size of IgG aggregates capable of perturbing liposomes. Native IgG was prepared at high concentrations (10–35 mg/ml PBS), aggregated in a water bath at 61.5°C for 10 min. Both the native and aggregated protein were subjected to density gradient centrifugation and all fractions tested at the same protein concentration for their ability to release $CrO_4^=$ and glucose from anionic liposomes. Two results emerged. First, release of marker induced by the corresponding fractions prepared from native IgG preparations was nearly identical to that produced by fractions of similar size prepared from intentionally aggregated

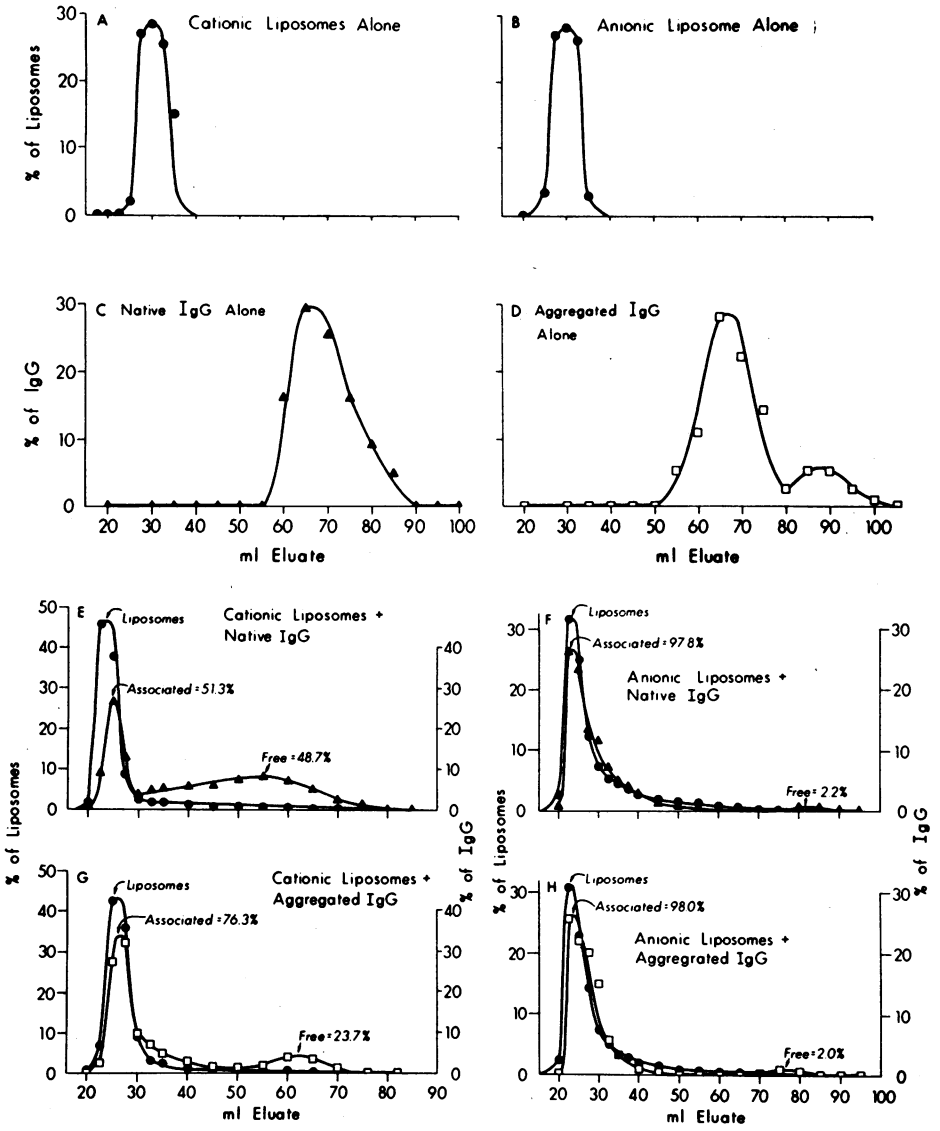


FIGURE 5 Column chromatography of liposomes and IgG using Sepharose 4B. The solutions (either liposomes alone, IgG alone, or liposomes incubated with 150 μg/ml IgG for 10 min) were eluted through Sepharose 4B with 0.145 M NaCl/KCl and collected at 2.5 ml or 5.0 ml samples. The results are expressed as the percent of intact liposomes recovered (apparent absorbance at 520 nm) and the percent of total IgG recovered (Lowry). (*A*) Cationic liposomes alone. (*B*) Anionic liposomes alone. (*C*) Native IgG alone. (*D*) Aggregated (10 min) IgG alone. (*E*) Separation of cationic liposomes and associated native IgG from free native IgG. (*F*) Separation of anionic liposomes and associated native IgG from free native IgG. (*G*) Separation of cationic liposomes and associated, aggregated IgG from free aggregated IgG. (*H*) Separation of anionic liposomes and associated, aggregated IgG from free aggregated IgG.

IgG. This indicated that native IgG contained aggregates and that native and aggregated fractions differed only in their relative *concentrations* of aggregates vs. monomers. Secondly, a distinctly greater release of anions or glucose from liposomes was observed after exposure to fractions II and III (Fig. 4), which contained 15–20-s aggregates than with the lighter species (fractions VI and VII or the more denatured pellet).

### Association of native and aggregated Ig with anionic and cationic liposomes

*Resolution with Sepharose 4B.* It has been shown previously that gel filtration (using Sephadex G-50 for anionic liposomes and Sephadex G-75 for cationic liposomes) could be used to resolve the spherules from glucose and from lysozyme which had not become membrane associated (40). An attempt to study the association between liposomes and IgG using the gels indicated that they could not be used to resolve the spherules from unassociated IgG. Subsequent experiments demonstrated that Sepharose 4B effectively resolved the lipid peak of both anionic and cationic liposomes from unassociated IgG (Fig. 5*A–D*).

*Interaction of anionic liposomes with native and aggregated IgG.* Washed liposomes (from Sephadex G-50) were incubated for 10 min with either native or aggregated IgG (150 μg/ml of liposomes) and then subjected to chromatography in 0.145 M NaCl/KCl on a Sepharose 4B column. Native samples had previously been centrifuged as above and only the lighter species were employed. Depending upon the degree of resolution desired, 2.5- or 5.0-ml samples of eluate were collected. Liposomes were detected in these samples by virtue of their apparent absorbance at 520 nm. In all cases the liposomes appeared after the void volume, and the integrity of their membranes was checked by boiling them to disrupt their lamellar configuration and then measuring release of trapped anions or glucose (40).

Approximately 98% of the recovered protein in both the native IgG-liposome and aggregated IgG-liposome mixtures became closely associated with the *anionic* membrane (Fig. 5*F* and *H*). These experiments demonstrated nonspecific association, presumably due to salt-like interactions between the anionic liposomes and cationic IgG. However, as in previous studies with the cationic protein melittin (*27*) and as shown above, salt-like interactions per se did not lead to marker release, which only followed exposure of anionic liposomes to *aggregated* IgG (see also Fig. 1).

*Interaction of cationic liposomes with native and aggregated IgG.* Washed, cationic liposomes (from Sephadex G-75) were incubated for 10 min with either native or aggregated IgG (150 μg/ml of liposomes), eluted from Sepharose 4B, and integrity of the lamellae was monitored as before (40). Again, native fractions were centrifuged to eliminate aggregates. In contrast to the salt-like associations between both native and aggregated IgG and *anionic* liposomes, positively-charged liposomes bound a portion of aggregated IgG preferentially (Fig. 5*E* and *G*). Whereas cationic liposomes became associated with about 50% of the available *native* protein, over 75% of the *aggregated* IgG had interacted with the bilayers. Since the difference represented specific association of the aggregated protein with membranes of similar surface charge, such associations cannot be ascribed to electrostatic interactions.

## DISCUSSION

The experiments described above demonstrate that liposomes release previously sequestered anions or glucose when exposed to immunoglobulins that have been heat-aggregated to mimic their configuration in immune reactions. The general results may be summarized as follows:

(*a*) Mild heat aggregation (10 min) of certain Ig confers upon these proteins the property of membrane perturbation as reflected by release of trapped markers from liposomes. $IgG_1$, $IgG_3$, and $IgG_4$ subclasses appear responsible for the activity of pooled IgG, whereas $IgG_2$ is inactive. $IgA_1$ is more active than $IgA_2$ while IgM is not rendered membrane-perturbing upon heating.

(*b*) This property of some immunoglobulins is not dependent upon the presence of cholesterol within the bilayers, nor upon the surface ζ-potential, operationally expressed as the molar ratio of long-chain anion or cation incorporated.

(*c*) Aggregated Fc fragments perturb liposomes, whereas Fab fragments do not enhance efflux of anions or glucose from liposomes.

(*d*) Increasing the time of aggregation (60 min to 24 h) destroys the membrane-perturbing activity of Ig.

(*e*) BSA cannot be rendered membrane-active by heat aggregation.

(*f*) Cationic liposomes preferentially associate with aggregated rather than with native IgG. Anionic liposomes associate with both native and aggregated protein by salt-like interactions which do not suffice to induce membrane perturbation, since only aggregated Ig release anions or glucose.

(*g*) Density gradient centrifugation demonstrated that the membrane-perturbing ability of heated IgG is due 15–20-s aggregates.

These findings may have direct bearing on certain effects of Ig on cell membranes, since they roughly parallel the interaction of various immunoglobulins with a variety of cells.

The presence of receptor sites for Ig on various types of white blood cells has been appreciated for several years (4, 11). Both monocytes and macrophages can bind Ig complexed with antigen or to erythrocytes, and IgG binds to membrane receptors more avidly than IgM. The binding of complexed Ig is inhibited by free $IgG_1$, $IgG_3$, and Fc, but not by $IgG_2$, $IgG_4$, IgA, or Fab (4, 41). Receptors on neutrophils (11) display similar orders of affinity towards these Ig. Henson, Johnson, and Spiegelberg (12) have recently shown that aggregated (but not native) Ig can provoke release of lysosomal enzymes from human neutrophils. Release of $\beta$-glucuronidase was induced by $IgG_1 >$, $IgG_3 >$, $IgG_4 >$, $IgG_2$, as well as by $IgA_1$ and $A_2$. Fc fragments were far more active than Fab, whereas IgM, IgD, and IgE were without effect on the cells. In contrast the cytoplasmic enzyme lactate dehydrogenase was not extruded, and it is clear that Ig stimulated the fusion of lysosomes with phagocytic vacuoles, leading to enzyme release by "regurgitation during feeding." The uptake and subsequent intracellular handling of immunoglobulins by phagocytes seems to depend, therefore, upon membrane contact followed by an as yet unspecified signal for internalization by the aggregated molecular or antigen-antibody complexes. This process may also govern platelet interactions with Ig, since heat aggregated, but not monomeric, IgG elicit release of serotonin from platelets (24); indeed monomeric proteins inhibited binding of aggregates. Furthermore, Arend and Mannik, studying the binding of soluble immune complexes to macrophages demonstrated greater inhibition of complex adherence by aggregated than by monomeric IgG (23). Ig molecules appear to interact with membranes through the Fc region (42). Aggregation and the formation of soluble immune complexes both produce alterations in the Fc portion of Ig thereby altering their biological activities (43). Neither neutrophils (12) nor liposomes interact with aggregated Fab fragments, but bind, or associate with the Fc portion of Ig. It therefore appears that phagocytes, like liposomes, possess receptor sites for certain classes of Ig and that aggregation enhances the association of immunoglobulins with natural and artificial membranes.

Strong associations between anionic liposomes and both native and aggregated IgG (as demonstrated by Sepharose 4B chromatography) are due to salt-like interactions between the anionic bilayers and cationic Ig as is the case also with melittin (27) or lysozyme (40).[3] A portion of aggregated Ig, however, became specifically associated with cationic liposomes. This association may therefore be due either to a conformational change conferred on individual aggregated molecules, or to the formation of multiple, lattice-like assemblies of Fc fragments capable of cooperative insertion into the bilayers. Either sort of interaction must be predominantly hydrophobic. H-bond mediated interactions between liposomes and surface contacts (44) are greatly enhanced by the presence of cholesterol (but are not required for immunoglobulin-induced perturbation), and salt-like interactions are not crucial for the selective action of aggregates since anionic liposomes bind aggregated and native species equally well.

It is more difficult to relate our findings to the interaction of antigens with surface immunoglobulins on B cells, in which the Fc portions are presumably embedded in the mosaic surface membrane. If heat aggregation induces changes in Ig which resemble those induced by antigens, it is possible, however, that upon addition of Ag, the Fc regions of intramembranous IgG molecules may undergo a similar conformational change. In this way a "message" would be generated to the interior of lymphocytes, triggering these cells into the production of antibodies. Whatever the mechanism of this series of events, it is clear that studies of interactions between aggregated immunoglobulins with purified phospholipids may provide insights into these important biological functions.

[3] Thus the bulk of small molecules are released in the presence of native macromolecules of opposite charge.


## ACKNOWLEDGMENTS

This work was aided by grants from the National Institutes of Health (AM-11949, HL-15140, and AM-02594), The Helen and Michael Shaffer Fund, the New York Heart Association, and The Whitehall Foundation.


## REFERENCES


1. Rabellino, E., S. Colon, H. M. Grey, and E. Unanue. 1971. Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. *J. Exp. Med.* **133**: 156.
2. Uhr, J. W., and E. S. Vitetta. 1973. Synthesis, biochemistry, and dynamics of cell surface immunoglobulin on lymphocytes. *Fed. Proc.* **32**: 35.
3. Lay, W. H., and V. Nussenzweig. 1968. Receptors for complement on leukocytes. *J. Exp. Med.* **128**: 991.
4. Huber, H., M. J. Polley, W. D. Linscott, H. H. Fudenberg, and H. J. Müller-Eberhard. 1968. Human monocytes: distinct receptor sites for the third component of complement and for immunoglobulin G. *Science (Wash. D. C.)*. **162**: 1281.
5. Henson, P. M. 1969. The adherence of leukocytes and platelets induced by fixed IgG antibody or complement. *Immunology*. **16**: 107.
6. LoBuglio, A. F., R. S. Cotran, and J. H. Jandl. 1967. Red cells coated with immunoglobulin G: binding and sphering by mononuclear cells in man. *Science (Wash. D. C.)*. **158**: 1582.
7. Huber, H., S. D. Douglas, and H. H. Fudenberg. 1969. The IgG receptor: an immunological marker for the characterization of mononuclear cells. *Immunology*. **17**: 7.
8. Abramson, N., E. W. Gelfand, F. S. Rosen, and J. H. Jandl. 1970. Specificity of monocyte receptor in man and monkey. *Clin. Res.* **18**: 397. (Abstr.)

9. Thrasher, S. G., and S. Cohen. 1971. Studies of the mechanism of binding of chemically modified cytophilic antibodies to macrophages. *J. Immunol.* **107**: 672.
10. Rabinovitch, M. 1967. Studies on the immunoglobulins which stimulate the ingestion of gluteraldehyde-treated red cells attached to macrophages. *J. Immunol.* **99**: 1115.
11. Messner, R. P., and J. Jelinek. 1970. Receptors for human γG-globulin on human neutrophils. *J. Clin. Invest.* **49**: 2165.
12. Henson, P. M., H. B. Johnson, and H. L. Spiegelberg. 1972. The release of granule enzymes from human neutrophils stimulated by aggregated immunoglobulins of different classes and subclasses. *J. Immunol.* **109**: 1182.
13. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. *J. Exp. Med.* **136**: 191.
14. Basten, A., J. F. A. P. Miller, J. Sprent, and J. Pye. 1972. A receptor for antibody on B lymphocytes I. Method of detection and functional significance. *J. Exp. Med.* **135**: 610 .
15. Fakhri, O., and J. R. Hobbs. 1972. Target cell death without added complement after cooperation of 7S antibodies with non-immune lymphocytes. *Nat. New Biol.* **235**: 177.
16. Huber, H., and H. H. Fudenberg. 1970. The interaction of monocytes and macrophages with immunoglobulins and complement. *Series Haematologica.* **3**: 160.
17. Berken, A., and B. Benacerraf. 1966. Properties of antibodies cytophilic for macrophages. *J. Exp. Med.* **123**: 119.
18. Liew, F. Y. 1971. The binding of the Fc fragment of guinea-pig cytophilic antibody to peritoneal macrophages. *Immunology.* **20**: 817.
19. Mantovani, B., M. Rabinovitch, and V. Nussenzweig. 1972. Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG). *J. Exp. Med.* **135**: 780.
20. Feinstein, A., and A. J. Rowe. 1965. Molecular mechanism of formation of an antigen-antibody complex. *Nature (Lond.).* **205**: 147.
21. Ishizaka, T., and K. Ishizaka. 1959. I. Skin reactive and complement-fixing properties of heat denatured gamma globulin. Biological activities of aggregated gamma globulin. *Proc. Soc. Exp. Biol. Med.* **101**: 845.
22. Ishizaka, K. 1963. Gamma globulin and molecular mechanisms in hypersensitivity reactions. *Prog. Allergy.* **7**: 32.
23. Arend, W. P., and M. Mannik. 1972. In vitro adherence of soluble immune complexes to macrophages. *J. Exp. Med.* **136**: 514.
24. Pfueller, S. L., and E. F. Luscher. 1972. The effects of aggregated immunoglobulins on human blood platelets in relation to their complement-fixing abilities. *J. Immunol.* **109**: 517.
25. Sessa, G., and G. Weissmann. 1968. Phospholipid spherules (liposomes) as a model for biological membranes. *J. Lipid Res.* **9**: 310.
26. Weissmann, G., G. Sessa, and S. Weissmann. 1965. Effect of steroids and "Triton X-100" on glucose-filled phospholipid/cholesterol structures. *Nature (Lond.).* **208**: 649.
27. Sessa, G., J. H. Freer, C. Colacicco, and G. Weissmann. 1969. Interaction of a lytic polypeptide. melittin, with membrane systems. *J. Biol. Chem.* **244**: 3575.
28. Sessa, G., and G. Weissmann. 1968. Effects of four components of the polyene antibiotic, filipin, on phospholipid spherules (liposomes) and erythrocytes. *J. Biol. Chem.* **243**: 4364.
29. Kinsky, S. C. 1972. Antibody-complement interaction with lipid model membranes. *Biochim. Biophys. Acta.* **265**: 1.
30. Lachmann, P. J., E. A. Munn, and G. Weissmann. 1970. Complement-mediated lysis of liposomes produced by the reactive lysis procedure. *Immunology.* **19**: 983.
31. Fahey, J. L., and A. P. Horbett. 1959. Human gamma globulin fractionation on anion exchange cellulose column. *J. Biol. Chem.* **234**: 2645.
32. Kunkel, H. G. 1954. Zone electrophoresis. *In* Methods of Biochemical Analysis. D. Glick, editor. Interscience Publishers, Inc., N. Y. **1**: 141.
33. Scheidegger, J. J. 1955. Une micro-methode del'immuno-electrophorese. *Int. Arch. Allergy Appl. Immunol.* **7**: 103.
34. Grey, H. M., and H. G. Kunkel. 1964. H Chain subgroups of myeloma proteins and normal 7S gammaglobulin. *J. Exp. Med.* **120**: 253.
35. Frangione, B., and E. C. Franklin. 1972. Chemical typing of immunoglobulin IgM, $IgA_1$, and $IgA_2$. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **20**: 321.
36. Franklin, E. C. 1960. Structural units of human 7S gamma globulin. *J. Clin. Invest.* **39**: 1933.
37. Keston, A. S. 1956. Determination of glucose by an enzymatic reagent. Abstracts of the 129th Meeting of the American Chemical Society. 31C.
38. Kaplan, J. H. 1972. Anion diffusion across artificial lipid membranes: the effects of lysozyme on anion diffusion from phospholipid liposomes. *Biochim. Biophys. Acta.* **290**: 339.
39. Warner, R. C., and M. Levy. 1958. Denaturation of bovine plasma albumin. II. Isolation of intermediates and mechanism of the reactions at pH 7. *J. Am. Chem. Soc.* **80**: 5735.
40. Sessa, G., and G. Weissmann. 1970. Incorporation of lysozyme into liposomes. A model for structure-linked latency. *J. Biol. Chem.* **245**: 3295.
41. Huber, H., and H. H. Fudenberg. 1968. Receptor sites of human monocytes for IgG. *Int. Arch. Allergy.* **34**: 18.
42. LoBuglio, A., and J. Rinehardt. 1970. In vitro and in vivo modification of human macrophage receptor for IgG globulin. *Clin. Res.* **18**: 409. (Abstr.)
43. Grossberg, A. L., G. Markus, and D. Pressmann. 1965. Changes in antibody conformation induced by hapten. *Proc. Natl. Acad. Sci. U. S. A.* **54**: 942.
44. Weissmann, G., and G. A. Rita. 1972. Molecular basis of gouty inflammation: interaction of monosodium urate crystals with lysosomes and liposomes. *Nat. New Biol.* **240**: 167.