Immunology, 1970, **19**, 715.

Passive Anaphylaxis in Mice with γG Antibodies

V. COMPETITIVE EFFECTS OF DIFFERENT IMMUNOGLOBULINS AND INHIBITION OF REACTIONS WITH ANTIGLOBULIN SERA

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(*Received* 21*st January* 1970)

Summary. Mouse antisera to DNP conjugates were fractionated on agar block electrophoresis, and each fraction was tested for ability to give anaphylactic reactions *in vivo* in mice and guinea-pigs, and *in vitro* on mouse mast cells. Concentrations of IgG_1 and IgG_2 globulin were measured for each fraction, and the results indicate that mouse IgG_1 globulin mediates both the *in vivo* PCA reaction in the mouse and the *in vitro* mast cell sensitization.

PCA reactions in guinea-pigs with mouse antisera are mediated only by electrophoretic fractions containing IgG_2 globulin. PCA reactions in guinea-pigs could be blocked by the addition of IgG_{2a} myeloma containing sera to the sensitizing anti-DNP preparation, but not by addition of IgG_{2b} or IgG_1 myeloma sera.

From six different IgG_1 myeloma sera tested on their capacity to block PCA reactions in mice, five showed strong inhibitory activity whereas the sixth was inactive. However, also IgG_{2a} and IgG_{2b} myelomas were found capable of blocking PCA reactions in mice. The passive *in vitro* sensitization of mouse mast cells by purified mouse anti-DNP antibodies could also be blocked by the addition of specific anti-IgG_2 sera. Possible interpretations of these findings are discussed.

INTRODUCTION

The immunoglobulins of most mammalian species have been subdivided into distinct classes on the basis of their different physicochemical, antigenic and biological properties (Franklin, 1964; Ovary, 1966; Bloch, 1967). Of particular value in studies on biological activities has been the property of some antibody molecules to provoke anaphylactic reactions in either homologous or heterologous species. With mouse IgG antibodies, only the IgG_1 class can elicit passive cutaneous anaphylaxis (PCA) in mouse skin (Nussenzweig, Merryman and Benacerraf, 1964; Barth and Fahey, 1965). When mouse myeloma proteins were tested for their ability to induce RPCA reactions in guinea-pigs (Ovary, Barth and Fahey, 1965) only IgG_{2a} myelomas were effective.

Previous studies with heterologous PCA reactions have indicated that large amounts of γ-globulins given intradermally with skin sensitizing antibodies, will block the local anaphylactic reaction induced by subsequent antigen administration. Gamma-globulins that can induce RPCA reactions are much more effective in exerting this blocking action (Ovary

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and Bier, 1953; Binaghi, 1968). This approach has now been applied to homologous PCA reactions using mouse myeloma sera as a source of large concentrations of the different mouse immunoglobulin classes.

Mouse anaphylactic reactions can also be induced *in vitro* with appropriate antisera and isolated mouse mast cells. In the present study, electrophoretic fractions of mouse antibodies were tested for their ability to passively sensitize mouse mast cells *in vitro*, and indicated that IgG_1 antibodies were responsible for this activity. The ability of various anti-immunoglobulin sera to block this reaction *in vitro* was also analysed.

MATERIALS AND METHODS

Mice. Adult female Swiss Webster (SW) mice were used as recipients for PCA reactions and as donors of peritoneal cell suspensions for *in vitro* tests. SW and A/HeJ mice (Jackson Laboratory, Bar Harbor, Maine) were immunized for the anti-hapten antibodies used for anaphylactic sensitization.

Guinea-pigs. Hartley strain albino guinea-pigs of both sexes weighing about 300 g were used as receptors for PCA reactions.

Antigens. Bovine serum albumin (BSA, Armour, Kankakee, Ill., lot A69908), bovine γ-globulin (BGG, Armour, lot X-30604), hens' egg albumin (Ov, Pentex, Inc., Kankakee, Ill., lot F-61) and haemocyanin (Hcy) from *Limulus polyphemus* (obtained in the laboratory as described by Campbell, Garvey, Cremer and Sussdorf, 1963), were dinitrophenylated according to methods previously reported (Eisen, Carsten and Belman, 1954; Ovary and Benacerraf, 1963). The following dinitrophenylated proteins were used: $DNP_{13}Ov$, $DNP_{37}BSA$, $DNP_{54}BGG$ and $DNP_{240}Hcy$. (The subscript numbers refer to the number of dinitrophenyl (DNP) groups per molecule of carrier protein. The molecular weight of haemocyanin was taken as 1×10^6 for this calculation.)

Mouse anti-DNP sera and antibodies. SW and A/J mice were immunized with either $DNP_{54}BGG$ or $DNP_{240}Hcy$ by repeated intraperitoneal injections of antigen in Freund's complete adjuvant (Difco Labs). The first injection contained 1 mg of antigen per mouse and subsequent booster injections 0·05 mg per mouse. Most of the data presented employed hyperimmune sera, collected after four to five antigen injections.

Anti-DNP antibodies were specifically isolated from these antisera by techniques previously described (Benacerraf, Ovary, Bloch and Franklin, 1963).

Electrophoresis of mouse anti-DNP serum. A sample of a hyperimmune A/J anti-DNP_{240} Hcy serum was subjected to electrophoresis in 1 per cent Ionagar in pH 8·2 veronal buffer on glass plates in a central trough 4 mm × 65 mm, for 5 hours at 20 mA and 10 V/cm. Serial 3 mm strips were then cut perpendicular to the direction of current flow, and the protein was eluted from the agar strips by soaking them in saline (buffered pH 7·5) overnight and then centrifuging at 35,000 rev/min for 1 hour. The supernatant fluids were then assayed for immunoglobulin content and antibody activity.

Antibody assays

PCA in guinea-pigs. PCA tests in guinea-pigs (groups of four to five) were made as previously described (Ovary, 1964) with 0·1 ml dilutions of test serum or fractions in saline for the intradermal injections, followed after a sensitization period of 4 hours by

intravenous challenge with 400 μg of $DNP_{37}BSA$ in 0·5 per cent Evans's blue solution. The magnitude of the PCA reactions was scored as previously described.

In blocking experiments on the PCA inhibitory capacity of mouse globulins, varying quantities of the test material were mixed with antibody dilutions just prior to the intradermal injection.

PCA in mice. PCA tests in SW mice were made as previously described (Vaz and Ovary, 1968a) with recipient mice receiving two intradermal injections of 0·03 ml one on either side of the shaved back. Challenge was made 2 hours later with 1 mg of $DNP_{13}Ov$ in 0·2 ml of a 0·5 per cent Evans's blue solution. As for blocking tests in guinea-pigs, the test sera and anti-DNP sera were mixed just prior to intradermal injection. All anti-DNP sera were preheated at 56° for 4 hours, to destroy reaginic activity.

Passive sensitization of mast cells. The anaphylactic histamine release from mouse peritoneal cells, containing thirty to sixty mast cells per mm^3 was induced after *in vitro* passive sensitization by incubation with purified mouse anti-DNP antibodies, and subsequent exposure to antigen. The techniques used have been previously described (Prouvost-Danon, Queiroz-Javierre and Silva Lima, 1966; Vaz and Ovary, 1968b). Briefly, aliquots from pooled cell suspensions collected from normal SW mice were exposed to 2 μg AbN/ml for 10 minutes at 37°, and then exposed to antigen (1 μg/ml $DNP_{37}BSA$) for a further 10 minutes. The amount of histamine in the supernatant and centrifuged cell pellets was then determined biologically with guinea-pig ileum preparations. The results were expressed as per cent of histamine released after deduction of the control release (which ranged from 5 to 13 per cent).

This technique was used in two sets of experiments: the first, testing for the presence of sensitizing antibodies in electrophoretic fractions, and the second investigating the ability of rabbit anti-mouse immunoglobulin sera to block the sensitization induced by a standard concentration of 2 μg AbN/ml of purified mouse anti-DNP antibodies.

In experiments assaying for the presence of sensitizing antibodies in the electrophoretic fractions, the cells were incubated with serial dilutions of the fractions and then challenged with 1 μg/ml $DNP_{37}BSA$. This was done because the magnitude of the anaphylactic release of histamine obtained with suspensions of mouse mast cells after passive sensitization *in vitro* is critically dependent on the relative proportions of antibody and antigen used for sensitization and challenge. If either are in excess a reduction in the percentage of the histamine released is observed (Vaz and Ovary, 1968b). Therefore, when an antibody solution of unknown concentration is to be treated by challenge with a fixed antigen concentration, a range of dilutions of the unknown solution must be treated in order to have a complete profile of the system passing through antibody excess, optimal proportions and antigen excess. In the present study the relative concentration of sensitizing antibodies present in the several fractions was represented as a function of the dilution of the fraction necessary to give maximal histamine release, i.e. in the test system used, with a fixed concentration of challenge antigen, the greater the antibody content of a particular fraction, the greater the dilution still effective to induce maximal histamine release.

In experiments studying the inhibitory effect of rabbit anti-mouse immunoglobulin sera on the sensitization of mast cells, the cells were incubated in mixtures of the purified mouse anti-DNP antibodies and dilutions of the rabbit antisera. After 10 minutes, challenge with antigen was made as above, and the inhibition of sensitization was expressed as the per cent of inhibition of histamine release induced in the control aliquots which contained mixtures of mouse antibodies and comparable dilutions of normal rabbit serum.

Quantitation of mouse immunoglobulins and assay of rabbit antimouse immunoglobulin sera

These assays were performed as previously reported (Herzenberg and Warner, 1968) with ^{125}I-labelled (Greenwood, Hunter and Glover, 1963) preparations of purified mouse myeloma proteins. Many of the antisera used were obtained through the generosity of Dr L. A. Herzenberg from previous joint collaborations.

Mouse myeloma sera and proteins

Serum samples were taken from mice with various transplantable plasma cell tumours at the time of maximal tumour growth, and were stored frozen at −20°. Several tumour lines with respective classes of myeloma protein were used and are mentioned in the tables.

RESULTS

ELECTROPHORETIC FRACTIONATION OF MOUSE ANTISERA

A sample of hyperimmune A/J anti-DNP_{240}Hcy serum was subjected to agar-block electrophoresis. Each fraction was then assayed for its content of IgG_1 and IgG_2 globulins (not discriminating between IgG_{2a} and IgG_{2b}) in appropriate specific inhibition of precipitation assays. Each fraction was also tested for sensitizing activity for both mouse and guinea-pig skin by PCA tests. In addition, the fractions were tested for their capacity to passively sensitize mouse mast cells *in vitro* for the anaphylactic release of histamine. The results are shown in Table 1 and graphically in Fig. 1. Table 1 also shows data from block electrophoresis of a purified mouse anti-DNP antibody preparation.

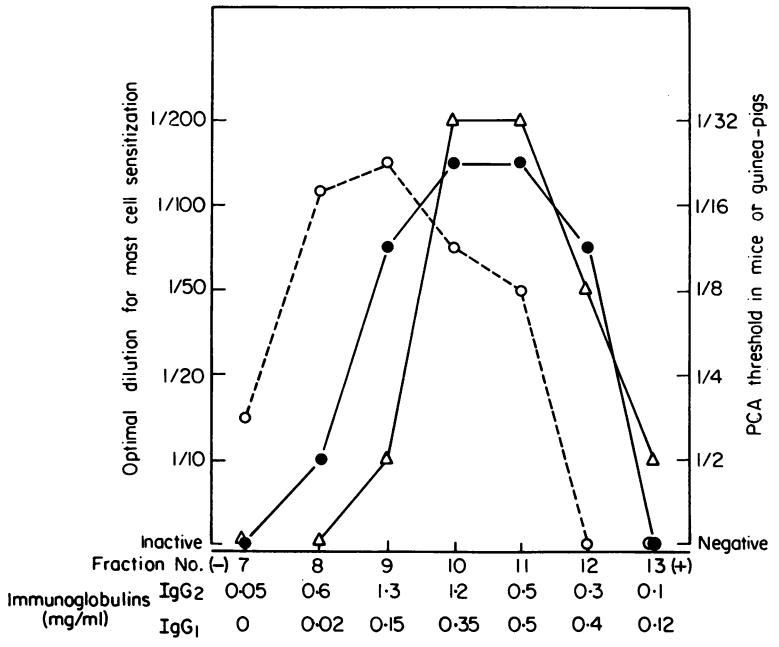

FIG. 1. Electrophoretic separation of mouse anti-DNP antiserum on agar block. The sample was applied at fraction 12 and electrophoresed with cathode to the left (lower numbers). The content of IgG_1 and IgG_2 globulin in each fraction is shown together with its antibody activity as measured in three biological assays. Mast cell sensitization, △; PCA in guinea-pigs, ○; PCA in mice, ●.

TABLE 1

BIOLOGICAL ACTIVITIES OF ELECTROPHORETIC FRACTIONS OF MOUSE ANTI-DNP ANTIBODIES

Starting material	Test systems	Fraction number* 7	8	9	10	11	12	13
Immune serum	PCA in guinea-pigs	3†	20	24	12	8	0	0
	PCA in mice	0‡	2	10	20	20	10	0
	Sensitization of mouse mast cells§	− (inactive)	− (inactive)	+ (1/10)	+++ (1/200)	+++ (1/200)	++ (1/50)	+ (1/10)
Purified Ab	PCA in guinea-pigs	4	30	30	30	20	5	0
	PCA in mice	0	0	5	10	10	5	0
	Sensitization of mouse mast cells	− (inactive)	− (inactive)	+ (1/10)	+++ (1/100)	+++ (1/100)	++ (1/50)	− (inactive)

* Origin in tube 12, cathode to the lowest number.

† Reciprocal of highest dilution giving threshold reaction; mean of four animals.

‡ Reciprocal of dilution giving mean PCA score of 2, in groups of five mice.

§ Signs − to +++ refer to magnitude of histamine release obtained after sensitization of mast cell suspensions by dilutions of the fractions; sign +++ represents release of about 40–60 per cent of total histamine which is approximately the maximal release obtainable by this method.

The numbers inside parentheses represent the dilution of the fraction in the cell suspension necessary to obtain maximum sensitization.

The electrophoretic separation of IgG_1 and IgG_2 globulins has not been complete, however, the IgG_1 globulin overlaps only with the anodal part of the IgG_2 globulin. The area of the IgG_2 globulins coincide with the area of PCA reactivity in guinea-pigs, whereas the IgG_1 globulin-containing fractions were the fractions with greater reactivity for PCA in mice. The ability to induce passive sensitization of mouse mast cells was also concentrated in the fractions containing most of the IgG_1 globulin. As explained in Methods, in the test system used, i.e. with a fixed concentration of antigen to challenge the mast cells, the greater the antibody content of a particular fraction, the greater the dilution of the fraction still effective to induce the anaphylactic release of histamine. As shown in Table 1 and Fig. 1, in order to induce optimal histamine release, 1/200 dilutions of fractions 10 and 11 were required. On the other hand, with fractions 9 and 13, dilutions equal or greater than 1/20 were inactive, whereas with a 1/10 dilution some histamine release could still be observed. In Table 1 both the dilution necessary for optimal histamine release, as well as the magnitude of the histamine release attained with each fraction are shown. The fractions with the peak of activity for mast cell sensitization are the fractions containing the bulk of sensitizing activity for mouse skin and also the bulk of IgG_1 globulins.

INHIBITION OF GUINEA-PIG PCA REACTIONS BY ADDITION OF MOUSE MYELOMA SERA

PCA reactions were performed in guinea-pigs with a standard pool of a hyperimmune A/J anti-DNP_{240}Hcy serum, and the reaction was developed with a challenge injection of 400 μg of DNP_{37}BSA. Serial dilutions of the anti-DNP serum were made in either saline or in a standard pool of normal mouse serum or mouse myeloma serum at a dilution of 1/5 or 1/10. A typical experiment is shown in Table 2 in which the anti-DNP serum gives a 50 per cent endpoint at the 1/800 dilution when the serum is diluted in saline or in the GPC-5 myeloma serum, but is reduced to 1/400 in normal mouse serum and to 1/100 in the GPC-7 myeloma serum.

TABLE 2

PCA IN GUINEA-PIGS WITH MOUSE ANTI-DNP-Hcy ANTISERUM DILUTION: INHIBITORY ACTIVITY OF MOUSE MYELOMA SERA

Diluent for mouse anti-DNP serum	Dilutions of mouse antiserum 25	80	100	200	400	800	1600
Saline	–*	–	–	12†	9	4	2
Normal mouse serum 1/10	–	–	10	8	5	0	–
GPC-7 (IgG_{2a}) myeloma serum 1/10	14	12	4	2	–	–	–
GPC-5 (IgG_{2b}) myeloma serum 1/5	–	–	12	8	6	4	–

* Not tested.
† mm PCA reaction, mean of four guinea-pigs.

The results for all myeloma sera tested are shown in Table 3. Three different IgG_{2b} myeloma sera (which contain lower than normal levels of IgG_{2a} globulin) fail to show any inhibition although the IgG_{2a} myeloma serum gives virtually complete inhibition even with a 1/80 dilution of the serum. Human γ-globulin, but not bovine γ-globulin is also capable of inhibiting the reaction. Three different IgG_1 myeloma sera also fail to block in guinea-pigs, even though they are extremely effective in inhibiting PCA reactions in mice (see next section).

TABLE 3

PCA IN GUINEA-PIGS WITH MOUSE ANTI-DNP-Hcy ANTISERUM: INHIBITORY ACTIVITY OF VARIOUS MOUSE MYELOMA SERA

Diluent for anti-DNP serum	Myeloma class	PCA titre*	Inhibitory activity
Saline	–	800	–
Bovine γ-globulin (10 mg/ml)	–	1600	–
Human γ-globulin (10 mg/ml)	–	100	++
Normal mouse serum 1/5	–	200	+
Normal mouse serum 1/10	–	400	±
Myeloma sera 1/5			
MPC-11	IgG_{2b}	800	–
GPC-5	IgG_{2b}	800	–
MPC-31	IgG_{2b}	800	–
HPC-22	IgG_1	400	±
HPC-39	IgG_1	800	–
HPC-32	IgG_1	800	–
GPC-7	IgG_{2a}	<50	+++
GPC-7 1/80	IgG_{2a}	50	+++

* Threshold dilution of anti-DNP serum capable of eliciting PCA reaction.

INHIBITION OF MOUSE PCA REACTIONS BY ADDITION OF MOUSE MYELOMA SERA

Sera from four different IgG_{2a} myeloma lines, four IgG_{2b} and six IgG_1 were tested for their ability to block PCA reactions in mice induced by an appropriate dilution of a mouse anti-DNP serum. The dilution of anti-DNP serum used was the minimum amount required to give a mean score of approximately 3+ (1/1000 dilution of this antiserum). The anti-DNP serum was mixed with an amount of myeloma serum just prior to intradermal injection with only one dilution of the anti-DNP serum being used. In all cases 7·5–15 μl of myeloma serum was injected per skin site in the total volume of 30 μl. The results in Fig. 2 show a titration of PCA activity against the amount of myeloma protein (using IgG_2 myeloma sera) per skin site added to the fixed amount of anti-DNP serum.

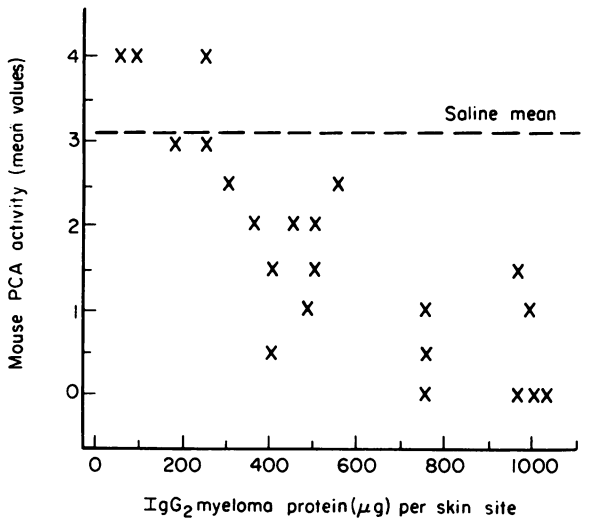

FIG. 2. Inhibitory activity of IgG_2 myeloma sera on PCA reactions in mice. Each point gives the mean PCA value for a group of mice receiving the indicated amount of IgG_2 globulin per skin site, admixed with a fixed amount of mouse anti-DNP antiserum.

Each point represents the mean value for a group of five mice and shows the amount of IgG_2 myeloma protein in the inoculum. The results in Table 4 give the mean data for the different groups of myeloma sera. For each serum the number of observations refers to the actual number of skin sites injected and pools the data from the different individual myeloma sera for each tumour line.

A slight but significant reduction in PCA activity is observed with normal mouse serum, and with MPC-25 (IgG_1) but not with MPC-1 (IgA) myeloma sera. A marked reduction in activity is observed with all other IgG_1, IgG_{2a} and IgG_{2b} myeloma sera.

TABLE 4

INHIBITION OF PCA REACTIONS IN MICE BY MOUSE MYELOMA SERA

Diluent for mouse antisera	No. of sites	Immunoglobulin class	Mean PCA score	Inhibitory activity
Saline	76	–	3·0*	–
Normal mouse sera	50	–	2·5	±†
MPC-1	20	IgA	3·0	–
MOPC-21	6	IgG_1	1·0	++
MPC-25	45		2·4	±
HPC-9	12		1·0	++
HPC-22	12		1·2	++
HPC-32	6		0	+++
HPC-39	6		0	+++
GPC-5	27	IgG_{2b}	0·2	+++
MPC-86	12		0	+++
MPC-31	30		0·5	+++
MPC-11	15		2·0	+
RPC-5	22	IgG_{2a}	1·5	+
GPC-7	21		0	+++
GPC-8	10		0·5	+++
5563	20		1·5	+

* In an arbitrary scale of magnitude ranging from 0 to 4, where 3+ represents a reaction of 12–15 mm in diameter, and 1+ a reaction of 5 mm in diameter.

† In relation to control values for antisera diluted in saline.

TABLE 5

EFFECT OF POLYVALENT RABBIT ANTI-MOUSE SERUM ON SENSITIZATION OF MOUSE MAST CELLS BY PURIFIED MOUSE ANTI-DNP ANTIBODIES

Mouse antibody	Rabbit serum	Dilution	Histamine*	
			Per cent released	Per cent inhibition
Anti-DNP-Hcy	Normal	1/5	54†	–
	Anti-mouse	1/5	4	93‡
	NIL	–	78	–
	Anti-mouse	1/5	13	83
		1/20	56	28
		1/80	66	16
Anti-DNP-BGG	NIL	–	82	–
	Anti-mouse	1/5	16	81
		1/20	42	49
		1/80	66	19

* Anaphylactic release of histamine after sensitization with 2 μg purified AbN/ml and challenge with 1 μg/ml $DNP_{37}BSA$.

† After deduction of 12 per cent control release.

‡ Expressed as observed decrease in histamine release.

INHIBITION BY ANTI-IMMUNOGLOBULIN SERA OF HISTAMINE RELEASE FROM SENSITIZED MAST CELLS *in vitro*

Normal mouse mast cells were incubated with purified mouse anti-DNP antibodies (2 μg AbN/ml) (containing approximately 20 per cent IgG_1 and 80 per cent IgG_2 antibody) in the presence of varying concentrations of a polyvalent rabbit anti-mouse globulin serum. The results in Table 5 show that addition of this antiserum at a concentration of 1/5 almost completely inhibits sensitization, and some inhibition is still evident at a 1/80 concentration.

Further tests were then performed with four different anti-immunoglobulin sera. Four to six dilutions of each serum were run in the assay, and the per cent of inhibition of histamine release was determined. These results were then graphed, and the appropriate dilution of antiserum required to give 50 per cent of inhibition was determined and is shown in Table 6. These antisera were also titrated against ^{125}I-labelled mouse myeloma proteins for their ability to give precipitation. The volume of serum required to precipitate 20 per cent of the labelled antigen (0·05 μg per tube) was determined and is also shown in Table 6. Serum anti-IgG (rabbit antiserum against mouse IgG) contains both anti-γ_1 H chain and anti-γ_2 H chain antibodies. Serum anti-IgG_2 is a rabbit anti-mouse IgG_2 Fc fragment serum and contains mainly anti-γ_2 H chain antibodies with only a trace of anti-γ_1 activity. Serum anti-IgG_{2a} is a mouse allotype (C57BL anti-NZB) antiserum specific for γ_{2a} H chains. These last three sera do not contain any anti-light chain antibody.

The polyvalent and anti-IgG_2 sera, although having only 10 per cent and 0·7 per cent respectively of the anti-γ_1 activity of serum anti-IgG, are nearly as efficient as their serum in causing inhibition of histamine release. Furthermore, the allotype anti-γ_{2a} serum is also capable of inhibition. This indicates that anti-γ_2 H chain antibodies are capable of inhibiting this mast cell sensitization.

DISCUSSION

The ability of homologous IgG antibodies to elicit PCA reactions in the mouse is thought to be a property only of the IgG_1 class of immunoglobulins (Nussenzweig *et al.*, 1964; Barth and Fahey, 1965). Our observations with electrophoretic fractions of mouse anti-DNP antibodies confirm this with fractions of known IgG_1 and IgG_2 content. This has been repeated in order to then compare the ability of these fractions to sensitize mouse skin (PCA reactions) and to passively sensitize mouse mast cells for anaphylaxis *in vitro*. The results in Fig. 1 and Table 1 clearly indicate that both the sensitizing activity for mouse skin and the ability to sensitize mast cells are associated with the migration of IgG_1 globulins. Since pre-heated anti-DNP antisera is devoid of reaginic activity (Mota and Peixoto, 1966; Revoltella and Ovary, 1969) it can be concluded that the *in vitro* mast cell sensitization assay measures the same IgG_1 antibody that mediates PCA reactions in the mouse.

The present results with PCA reactions in guinea-pigs are in complete agreement with previous observations indicating that only mouse IgG_{2a} globulin can mediate this reaction (Ovary *et al.*, 1965). Using the reverse PCA technique two additional purified IgG_{2a} myelomas (GPC-7 and HPC-3) have also been found to elicit this reaction.

Previous studies with heterologous immunoglobulin fractions have shown that PCA reactions in guinea-pigs induced by small amounts of sensitizing antibodies, can be blocked

TABLE 6

ANAPHYLACTIC RELEASE OF HISTAMINE FROM MOUSE MAST CELLS PASSIVELY SENSITIZED *in vitro* WITH PURIFIED MOUSE ANTI-DNP ANTIBODIES: INHIBITORY EFFECT OF RABBIT ANTI-MOUSE IMMUNOGLOBULIN SERA

Rabbit anti-immunoglobulin sera	Dilution effective for 50 per cent inhibition of the histamine release	Specificity of inhibiting antisera						
		IgG_1	IgG_{2a}	IgG_{2b}	IgG_1	IgG_{2a}	IgG_{2b}	IgA
Anti-IgG	1/300*	100†	100	100	0·02‡	0·03	0·03	> 5·0
Anti-mouse serum	1/150	10	50	75	0·21	0·06	0·04	0·25
Anti-IgG_2	1/150	0·7	37	100	3·1	0·08	0·03	> 5·0
Anti-IgG_{2a}	1/60	0	43	0	5·0	0·07	5·0	> 5·0
Normal rabbit serum	inactive at 1/5	–§	–	–	–	–	–	–

* Values interpolated from experiments measuring the inhibitory activity of serial dilutions of the anti-immunoglobulin antisera upon a standard histamine releasing system consisting of: 2 μg AbN/ml of purified mouse anti-DNP antibodies plus 1 μg/ml $DNP_{37}BSA$.

† Specificity of the rabbit antisera for various mouse immunoglobulin classes expressed as a percentage of the efficiency of the most active antiserum (anti-IgG). The same data are shown in ‡ with their actual values in the immunoglobulin assays.

‡ μl of antisera necessary to precipitate 20 per cent of 0·05 μg of [^{125}I]myeloma protein of the appropriate immunoglobulin class.

§ Not tested.

by the addition of large amounts of normal γ-globulins (Ovary and Bier, 1953). Only γ-globulins from species that can induce RPCA reactions in guinea-pigs were capable of this blocking and this is demonstrated in Table 3 in that HGG but not BGG would block the reaction. The results in this paper extend this blocking type of experiment to the addition of mouse immunoglobulins—using mouse myeloma sera, and studying the blocking both in mouse and guinea-pig. The results in guinea-pigs are in full agreement with the previous results for blocking with heterologous γ-globulins in that only the immunoglobulin class capable of mediating RPCA reactions (IgG_{2a}) was capable of blocking sensitization with an anti-hapten antibody. Some reduction in PCA activity was also obtained when the anti-DNP serum was diluted in normal mouse serum, and even this degree of reduction was not observed with the IgG_{2b} myeloma sera. This would be explicable in terms of the IgG_{2a} in normal serum giving some blocking, and this normal level is usually reduced when a myeloma of another class is present.

In contrast to these results in guinea-pigs, the observations made on anaphylactic reactions in mice, both *in vivo* and *in vitro*, indicated that although IgG_1 antibodies are mediating the anaphylactic sensitization, other classes of immunoglobulins may interfere with it. Thus, although homologous IgG_1 globulin mediates mouse PCA, both IgG_{2a} and IgG_{2b} myeloma containing sera as well as IgG_1 myeloma sera were capable of inhibiting the reaction when present in large amounts. In two instances, MPC-11 (IgG_{2b}) and GPC-7 (IgG_{2a}) blocking has also been demonstrated with the isolated myeloma protein. Furthermore, anti-IgG_2 specific antisera could block the sensitization *in vitro* of mouse mast cells by mouse anti-DNP antibodies.

It has recently been reported (Mota, Wong and Sadun, 1968) that pretreatment of mouse antisera containing both IgG_1 and reaginic types of sensitizing antibody, with specific anti-IgG_1 sera will completely abolish the capacity of the antisera to evoke PCA reactions with short (2–4 hours) sensitization periods, without affecting the sensitization induced by the reaginic antibodies. Specific anti-IgG_{2a} or anti-IgG_{2b} sera were ineffective. Our results are in apparent contradiction with these findings and three alternative explanations may be proposed to explain these discrepancies. (1) The cytophilic binding properties of homologous IgG_2 globulins is well known; previous studies have shown that the bulk of the IgG_2 fraction can specifically bind to macrophage receptors (Berken and Benacerraf, 1966). If we presume that the mast cell also carries a similar receptor, it would then be expected that large amounts of IgG_2 protein might give a degree of binding which was sufficient to block the attachment of the IgG_1 antibody–antigen complex. This would infer that the type of binding was different from antibody–antigen complex binding, and did not induce histamine release. The *in vitro* blocking of anti-IgG_2 specific antisera can also be explained by cytophilic binding of IgG_2 globulins to the surface of mast cells. These globulins would bind the rabbit anti-IgG_2 antibody and prevent the subsequent attachment of the mouse IgG_1-anti-DNP antibody by steric hindrance. As this sytem does not contain complement, no direct cytotoxic action of the anti-IgG_2 serum would be expected. Preliminary experiments utilizing sheep erythrocytes coated with DNP-protein and then reacted with mouse anti-DNP IgG_2 antibodies have shown that these erythrocytes are capable of forming rosettes around washed mouse mast cells (Tigelaar, R. E., Vaz, N. and Ovary, Z., to be published). (2) Previous experiments (Vaz and Ovary, 1968b) have indicated that the histamine release induced in mouse mast cells after sensitization by homologous antibodies is mediated by antibody–antigen complexes, and does not depend on antibody fixation to mast cells before the antibody–

antigen interaction. If free antibody were to be bound to mast cells before antibody–antigen interaction, then the reaction should be capable of inhibition by anti-IgG_1 antibodies alone, and not by other anti-immunoglobulin sera. However, the results in Table 6 clearly indicate that anti-IgG_2 antibodies are also fully capable of inhibiting this reaction. This experiment was performed with a purified anti-DNP antibody preparation which contained both IgG_1 and IgG_2 antibodies in the ratio of 1 : 4. It is probable, therefore, that when the antibody preparation is exposed to $DNP_{37}BSA$ most of the antibody–antigen complexes formed contain both IgG_1 and IgG_2 antibodies. Hence the specific anti-mouse IgG_2 antibody would be able to block the anaphylactic reaction through binding to IgG_2 molecules present in (IgG_2–$DNP_{37}BSA$–IgG_1) complexes, and thus making the IgG_1 moiety of the complex unavailable to mast cells. Thus, although histamine release is mediated exclusively by IgG_1 antibodies, anti-IgG_2 antibody can block the reaction, in turn inferring that the reaction is only mediated by complexes and not by free antibody binding to the mast cells. (3) Since a complete separation of IgG_1 and IgG_2 anti-DNP antibodies has not been achieved in electrophoresis, it would be theoretically possible that a population of relatively fast IgG_2 antibodies could mediate anaphylactic reactions in mice and would therefore be blocked by anti-IgG_2 antibodies.

It has also been proposed (Binaghi, 1968) that the inhibition of anaphylactic sensitization of concentrated γ-globulin solutions is due to protein–protein interaction, between the sensitizing antibodies and the normal γ-globulins, occurring in solution, and not to competition for cellular receptors. In the present experiments IgG_{2b} and IgG_1 myeloma sera which were able to block PCA reactions in mouse skin were not able to block PCA reactions in guinea-pig skin induced with the same mouse anti-DNP serum. It would then have to be necessary to assume that protein–protein interactions may occur affecting the IgG_1 portion (PCA in mice), but not the IgG_{2a} (PCA in guinea-pigs) portion of the mouse sensitizing antibodies.

Regardless of this question, it would be expected that, as in PCA reactions in guinea-pigs, a myeloma of the class which induces the PCA reaction should be capable of blocking the reaction. Of the six IgG_1 myeloma sera tested only one failed to significantly block the reaction. Further studies are necessary with more IgG_1 myeloma proteins to determine whether the non-blocking protein indicates the existence of a subclass of mouse IgG_1 globulin lacking the sensitizing activity for homologous tissue.

ACKNOWLEDGMENTS

We are grateful to Mr Csaba de Szalay for extremely competent technical assistance.

This work was supported by National Institutes of Health Grant AI-03075-11 and by the Health Research Council, City of New York, under Contract I-140. This work was also carried out with a travel fellowship from the Conselho Nacional de Pesquisas, Brazil (N.M.V.) and a Wellcome Trust Travel Fellowship (N.L.W.). One of us (N.L.W.) is a Fellow of the Helen Hay Whitney Foundation; and Z.O. is a Health Research Council Career Scientist of the City of New York.

REFERENCES

BARTH, W. F. and FAHEY, J. L. (1965). 'Heterologous and homologous skin sensitizing activities of mouse 7S γ1 and 7S γ2 globulins.' *Nature (Lond.)*, **206**, 730.

BENACERRAF, B., OVARY, Z., BLOCH, K. J. and FRANKLIN, E. C. (1963). 'Properties of guinea pig 7S antibodies. I. Electrophoretic separation of two types of guinea pig 7S antibodies.' *J. exp. Med.*, **117**, 937.

BERKEN, A. and BENACERRAF, B. (1966). 'Properties of antibodies cytophilic for macrophages.' *J. exp. Med.*, **123**, 119.

BINAGHI, R. A. (1968). 'The sensitization of tissues, and interference of non-specific gamma-globulin.' *Biochemistry of the Acute Allergic Reactions* (Ed. by K. F. Austen and E. L. Becker), p. 53. Blackwell Scientific Publications, Oxford.

BLOCH, K. J. (1967). 'The anaphylactic antibodies of mammals including man.' *Progr. Allergy*, **10**, 84.

CAMPBELL, D. H., GARVEY, J. S., CREMER, N. E. and SUSSDORF, D. H. (1963). *Methods in Immunology*, p. 69. Benjamin, New York.

EISEN, H. N., CARSTEN, M. E. and BELMAN, S. (1954). 'Studies of hypersensitivity to low molecular weight substances. III. The 2,4-Dinitrophenyl group as a determinant in the precipitin reaction.' *J. Immunol.*, **73**, 296.

FRANKLIN, E. C. (1964). 'The immune globulins—their structure and function and some techniques for their isolation.' *Progr. Allergy*, **8**, 58.

GREENWOOD, F. C., HUNTER, W. M. and GLOVER, J. S. (1963). 'The preparation of I^{131} labelled human growth hormone of high specific radioactivity.' *Biochem. J.*, **89**, 114.

HERZENBERG, L. A. and WARNER, N. L. (1968). *Genetic Control of Mouse Immunoglobulins in Regulation of the Antibody Response* (Ed. by B. Cinader), p. 322. Thomas, Springfield, Illinois.

MOTA, I. and PEIXOTO, J. M. (1966). 'A skin sensitizing and thermolabile antibody in the mouse.' *Life Sci.*, **5**, 1723.

MOTA, I., WONG, D. and SADUN, E. H. (1968). 'Mouse homocytotropic antibodies. I. Specific differentiation between mouse 7S γ1 and mouse reagin-like antibodies.' *Life Sci.*, **7** (part II), 1289.

NUSSENZWEIG, R. S., MERRYMAN, C. and BENACERRAF, B. (1964). 'Electrophoretic separation and properties of mouse anti-hapten antibodies involved in passive cutaneous anaphylaxis and passive hemolysis.' *J. exp. Med.*, **120**, 315.

OVARY, Z. (1964). 'Passive cutaneous anaphylaxis.' *Immunological Methods; C.I.O.M.S. Symposium* (Ed. by J. F. Ackroyd), p. 259, Blackwell Scientific Publications, Oxford.

OVARY, Z. (1966). 'The structure of various immunoglobulins and their biological activities.' *Ann. N.Y. Acad. Sci.*, **129**, 776.

OVARY, Z., BARTH, W. F. and FAHEY, J. L. (1965). 'The immunoglobulins of mice. III. Skin sensitizing activity of mouse immunoglobulins.' *J. Immunol.*, **94**, 410.

OVARY, Z. and BENACERRAF, B. (1963). 'Immunological specificity of the secondary response with dinitrophenylated proteins.' *Proc. Soc. Exp. Biol. (N.Y.)*, **114**, 72.

OVARY, Z. and BIER, O. G. (1953). 'Action empêchante du serum normal de lapin sur l'anaphylaxie cutanée passive du cobaye.' *Ann. Inst. Pasteur*, **84**, 443.

PROUVOST-DANON, A., QUEIROZ-JAVIERRE, M. and SILVA LIMA, M. (1966). 'Passive anaphylactic reaction in mouse peritoneal mast cells *in vitro*.' *Life Sci.*, **5**, 1751.

REVOLTELLA, R. and OVARY, Z. (1969). 'Reaginic antibody production in different mouse strains.' *Immunology*, **17**, 45.

VAZ, N. M. and OVARY, Z. (1968a). 'Passive anaphylaxis in mice with γG antibodies. I. PCA and RPCA reactions with homologous and heterologous antibodies.' *J. Immunol.*, **100**, 169.

VAZ, N. M. and OVARY, Z. (1968b). 'Passive anaphylaxis in mice with γG antibodies. III. Release of histamine from mast cells by homologous antibodies.' *J. Immunol.*, **100**, 1014.