Clin. exp. Immunol. (1970) **6**, 1–12.

SYMPOSIUM ON IgE AND ATOPIC ALLERGY

(Joint Meeting of the Dutch and British Societies for Immunology, Amsterdam, June 1969)

IMMUNOCHEMICAL MECHANISMS OF IMMEDIATE-TYPE HYPERSENSITIVITY REACTIONS

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(*Received* 19 *July* 1969)

SUMMARY

The properties of the main types of anaphylactic antibodies are compared. Their modes of action are considered in immunochemical terms in relation to recent findings about the mechanism of immediate hypersensitivity reactions in humans, which have been obtained from studies facilitated by the use of a myeloma form of IgE.

It is suggested that the combination of cell bound anaphylactic antibody with specific antigen (allergen) induces a conformational change within the Fc region of the antibody molecule, and this initiates a critical reaction at the cell surface which is responsible for the activation of the series of enzyme reactions involved in the release of histamine and other vasoactive amines.

INTRODUCTION

The elucidation of the mechanism of immediate-hypersensitivity reactions involves not only the isolation and characterization of the tissue sensitizing antibodies, but also the identification of their target cells and the delineation of the secondary role played by other cells and by humoral factors. It is necessary, also, to define the types of pharmacologically active agents produced on antigen challenge and to explain how these substances mediate the types of reactions which are referred to collectively as 'anaphylactic'. Right at the outset it is important to underline the fact that the finally observed response, whether it be an increase in cutaneous capillary permeability or a contraction of a guinea-pig ileum preparation, can be evoked in several different ways. The objective of this review is the assessment of the current state of knowledge about the immunochemical basis of immediate-type hypersensitivity reactions. In tackling this subject I shall pay particular attention to three aspects:

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(1) The nature of the antibody types involved, and the characteristics which distinguish them from each other and from other types of antibody.

(2) The manner in which they interact with their target cells.

(3) The nature of the reaction resulting from the combination of antigen (allergen) with cell bound antibody.

Turning first to the sensitizing antibodies, there is probably little need for me to describe the exciting advances which have been made in this field in the last few years. We can now discard the term 'reagin', as IgE has achieved respectability mainly as a result of the inexhaustible efforts of the Ishizaka group in Denver and the discovery of a myeloma counterpart by Johansson & Bennich (1967). I do not want to anticipate what Dr Ishizaka and Dr Johansson have to say about their work, later in this symposium. I shall, therefore, be concentrating on my own contributions, which have been concerned recently with the use of the myeloma IgE (initially referred to as IgND) to investigate the mechanism of binding of IgE antibodies to isologous and closely related heterologous tissues. Before going on to this subject, however, it is worth taking a broader view of the field and considering the nature and function of tissue sensitizing antibodies in general; whether they are produced spontaneously in humans or experimentally in animals.

COMPARISON OF PROPERTIES OF ANAPHYLACTIC ANTIBODIES

Inevitably, the recent work which has permitted the association of human tissue-sensitizing antibodies with a new immunoglobulin class (IgE) has facilitated the identification of a reagin-type antibody, with similar biological and physico-chemical properties in many other species such as monkeys, dogs, rabbits, rats, mice and sheep following immunization with a range of different antigens or infestation with certain parasites.

TABLE 1. Properties of three major types of anaphylactic antibodies

Property	Reagin-like	Guinea-pig γ_1-type	Guinea-pig γ_2-type
Tissue sensitized	Isologous	Isologous	Heterologous
Optimum sensitization time (hr)	50–80	2–4	2–4
Persistence in skin	4 weeks	1–2 days	1–2 days
Minimum sensitive dose (μg)	10^{-5}	10^{-2}	10^{-2}
Antigen-coated RBC agglutination	+	+	+
Complement fixability	−	−	+
Heat (56°C for 30 min)	Labile	Stable	Stable
Placental transmission	−	+	+
Electrophoretic mobility	γ_1	γ_1	γ_2
Sedimentation rate	8S	7S	7S

It is fascinating to find that such antibodies which are produced under somewhat different sensitization conditions to the human IgE antibodies found in the sera of patients with hay fever and asthma, possess very similar properties to reagins although they do, of course, often show only a transitory appearance in the serum. Nevertheless, even though these

antibodies have not yet been characterized to the same extent, I feel that it is justifiable to refer to them tentatively as IgE type. A complication, however, as far as the animal systems are concerned, is the chance of confusing such antibodies with another type of isologous tissue sensitizing antibody which appears to belong to a different immunoglobulin class. I am, of course, referring to the guinea-pig γ_1-type of antibody, which like IgE antibodies moves in the fast γ region on electrophoresis and possibly becomes fixed to the surface of mast cells. There are, however, several ways in which IgE and γ_1-type antibodies can be distinguished from each other, as is shown in Table 1 (based on data from the literature). For practical purposes, the two most useful criteria would seem to be the marked difference in the time required for them to sensitize isologous tissue optimally, and their differing susceptibility to heat treatment at 56°C. It has been shown that the IgE antibody activity of human allergic serum, whether measured by Prausnitz–Küstner (P–K) testing *in vivo* or passive leucocyte sensitization *in vitro*, is drastically reduced (to approximately 20% of the original activity) by heating the serum at 50°C for 30 min; whilst all activity was destroyed by heating for a further 30 min (Stanworth & Kuhns, 1963). Obviously the time needed to destroy all activity by heating at this temperature will depend on the level of IgE antibody in the allergic serum, but this should not approach the appreciably larger time of heating (i.e. several hours) needed to effect the activity of antibodies of the guinea-pig γ_1-type. Recently published work suggests that a similar type of 'homocytotropic' antibody can also be produced in other species such as mice and rats, but there are indications that these differ from the guinea-pig γ_1-type in certain respects.

Of course, it would be naive to assume that a rigid analogy obtains between the Ig classes of various animal species. (The differences in sub-class composition of the IgG of human and other species suggests otherwise, to quote but one example.) Nevertheless, the identification of a second anaphylactic antibody in the animal species mentioned should encourage attempts to demonstrate a similar immunoglobulin in allergic human sera. Could this account for the reported discrepancies between the activities of the sera of certain types of allergic patient (e.g. those sensitive to certain drugs), as measured by accepted IgE assays, and the observed clinical state of such patients? I suppose it is possible that sensitizing antibody of the IgG type, say, is responsible for the basophil degranulation activity of these patients' sera, as this is demonstrable under conditions which are far from favourable for antigen-induced histamine release from IgE-sensitized cells.

This illustrates the value of using animal model systems to obtain better understanding of the mechanism of human hypersensitivity reactions. Such systems are, of course, proving particularly useful in attempts to identify the target cells at the seat of anaphylactic reactions, for it is hardly justifiable to render a human leukopenic by treatment with nitrogen mustard or to decomplement him with cobra venom. This sort of approach is being adopted by Austen and Bloch, and associates, in their interesting studies of the anaphylactic antibodies of the rat. The summary of their recent published findings (Morse, Austen & Bloch, 1969), shown in Fig. 1, brings out the important point that the two types of 'homocytotropic' antibody induced in rats (i.e. IgE and IgG_a) interact with different target cells resulting in the production of two different pharmacologically active agents (histamine and SRS-A). Furthermore, it seems that the heat-stable IgG_a type antibody (anti-DNP) may itself interact with two different target cells. As the authors mention, however, it is still possible that an as yet unknown antibody type with similar physico-chemical properties to the IgG_a, is responsible for one of these reactions.

It is conceivable that the lower pathway shown in Fig. 1 (i.e. involving the participation of host complement and leucocyte lysosomal enzymes) is initiated by an isologous tissue-sensitizing IgG antibody with a similar mode of action to the heterologous tissue-sensitizing guinea-pig γ_2-type (referred to in the classification in Table 1). This suggestion is prompted by the observations of Movat *et al.* (1967) that some heterologous (γ_2-type) hyperimmune antibodies mediate passive cutaneous anaphylaxis (PCA) reactions in guinea-pigs primarily by release of lysosomal material from PMN leucocytes. Lovett & Movat (1966) have suggested that this process might occur as a result of phagocytosis of microprecipitates of antigen and antibody facilitated by host complement.

It is interesting that the other pathway of the rat IgG_a antibody resembles that of the guinea-pig γ_1-type. The observation that disodium cromoglycate suppresses histamine release mediated by either the heat-labile IgE-type or the heat-stable IgG_a-type of rat anti-

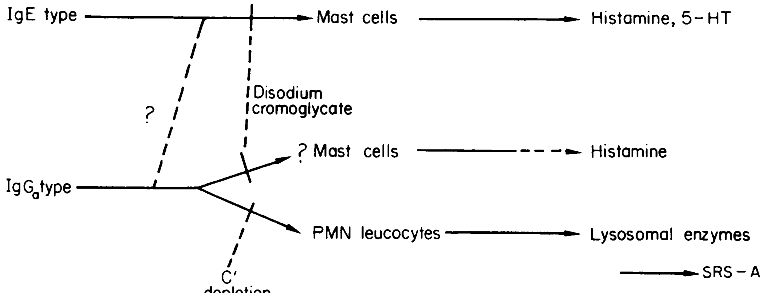

FIG. 1. Postulated modes of action of rat tissue sensitizing antibodies (based on data of Morse, Austen & Bloch, 1969).

body has been interpreted as evidence that these two pathways share a similar or common step. This raises the question, of course, as to whether a similar relationship obtains between IgE and γ_1-type anaphylactic antibodies *in other species*. In fact, can this interesting work on the rat systems be taken as a prototype? Here one might be treading on dangerous ground, as other types of mechanisms have already been described in other animal systems (e.g. involving *in vitro* release from rabbit platelets induced by antigen-treated lymphocytes, as described by Barbaro & Schoenbechler, 1969). Nevertheless, it should be recognized that the findings of Morse *et al.* (1969) are based on passive sensitization and antigen challenge within the whole animal, in contrast to the use of isolated cell or tissue preparations which might lack co-factors which are essential for some types of anaphylactic reaction.

Hence it seems that *there are three major types of antibody capable of mediating immediate hypersensitivity reactions.* Admittedly, one of these (the γ_2-type) is not anaphylactic within its species of origin, but this does not necessarily mean that its study will not throw further light on the roles of the other two ('homocytotropic') types; nor is it necessary at this stage to become involved in the topical debate as to whether the non-IgE types of antibody behave like the IgE-type in becoming attached to the target cell surface prior to interaction with antigen (although I consider this to be quite probable).

MODE OF INTERACTION OF ANAPHYLACTIC ANTIBODIES WITH CELLS

The question which I am now going to consider is that of the identification of the structure(s)

within the antibody molecules responsible for interactions with the target cells. The early direct and reverse PCA tests in guinea-pigs, using heterologous immunoglobulins, pointed to the involvement of the Fc region in tissue attachment. For instance, of the then known human immunoglobulin classes only the IgG (in contrast to the IgA or IgM) proved capable of sensitizing guinea-pig skin (Franklin & Ovary, 1963). By similar reasoning, Ovary, Benacerraf & Bloch (1963) concluded that guinea-pig γ_1-type antibodies bound to isologous tissue through binding sites located in their Fc regions whereas the guinea-pig γ_2-type antibodies (possessing different, but nevertheless structurally related, Fc determinants) were considered to lack such receptors. As is now well known, Ovary & Karush (1961) later obtained more definitive evidence, as far as heterologous tissue sensitization was concerned, in showing that removal of the Fc region of rabbit IgG antibody molecules by peptic cleavage destroyed their ability to fix to guinea-pig skin and, conversely, Fc fragments obtained from such antibodies by papain cleavage were able to inhibit the induction of PCA reactions by the intact antibody molecules.

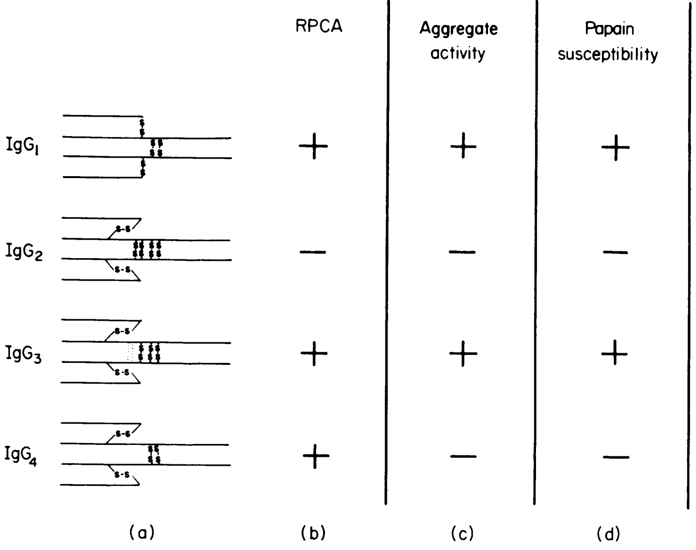

FIG. 2. Comparison of skin reactivities and structural characteristics of the four sub-classes of human IgG based on data of: (a) Frangione *et al.* (1969); (b) Terry (1965); (c) Ishizaka *et al.* (1967); (d) Jefferis *et al.* (1968); Gergely (personal communication 1969).

Despite these fundamental observations, however, it is surprising to find that not all γG-type antibodies produced in a particular species (e.g. human and rabbit) are capable of inducing PCA reactions in guinea-pigs. For example, human anti-dextran produced only weak *in vivo* reactions and showed only poor sensitization of intestine *in vitro*. Moreover, it has not proved possible to sensitize guinea-pigs' skin with IgG antibodies raised in certain species (such as horse, sheep and cow) irrespective of the immunizing antigen; nor are the γG-globulins of these species capable of effecting reverse passive cutaneous anaphylaxis (RPCA) reactions in guinea-pigs. A clue to the immunochemical explanation of these observations has been provided from the recent studies of Yount *et al.* (1968), which have revealed that humans immunized with dextran and other polysaccharide antigens produce

antibodies confined to a single IgG sub-class namely the IgG2. This is the only one of the four sub-classes of human IgG which was found (Terry, 1965) to be incapable of eliciting a RPCA reaction as is shown in Fig. 2 (abridged from Frangione, Milstein & Pink, 1969). The immediate skin reactivity of aggregated preparations of each sub-class in normal guinea-pigs (determined by Ishizaka *et al.*, 1967) is also included for comparison, as is also the papain susceptibility (4 hr digestion, 37°C, in the absence of cysteine) of each IgG sub-class (determined independently by Jefferis *et al.*, 1968, and Gergely, personal communication, 1969).

The outstanding resistance of the IgG2 sub-class to papain cleavage, even in the presence of cysteine (0·01 M), can be attributed to the multiple interchain S–S bridges within the hinge region which would be expected to have a profound influence on the flexibility of the molecule and to restrict the access of the enzyme. For a similar reason the binding of this sub-class of IgG globulin on to receptor sites on the target cell surface (in the guinea-pig skin) could be impeded.

We are exploiting the relationship between immunoglobulin structure and susceptibility to proteolysis, in attempts to differentiate sub-classses within the γG-globulin fraction of other species (for which myeloma counterparts do not exist). If, for example, we compare the papain susceptibility of γG-globulin preparations isolated by batch chromatography on DEAE-cellulose (Stanworth, 1960) using 0·01 M phosphate buffer (pH 6·5) we obtain the results shown in Table 2. The point to which I want to direct attention is the finding that the

TABLE 2. Comparison of skin reactivities of IgG of various species with their composition as reflected by their susceptibility to digestion by papain

Species	PCA in guinea-pigs	% resistant to papain (4 hr, 37°)
Human	+	40
Monkey	+	51
Rabbit	+	66
Horse	–	95
Sheep	–	90
Cow	–	95

γG-globulins of those species which fail to evoke PCA (or RPCA) reactions in guinea-pigs are substantially resistant to digestion with papain (4 hr, 37°C in the absence of cysteine). I suggest that these findings begin to offer an explanation of the inability of the γG-globulins of the horse, sheep and cow to evoke PCA reactions in guinea-pigs. It seems likely that these species are incapable of producing γG-globulin sub-classes with the appropriate structures within their Fc regions necessary for binding to the target cell receptors. In other words, these species tend to produce γG-globulins with critical structural characteristics similar to those found in the unreactive human IgG2 sub-class.

IgE-cell interaction

An obvious question to be answered was whether IgE antibodies bind to their target cells in a similar manner to the mode of combination of IgG antibodies to heterologous tissue.

Our original P–K-inhibition tests with whole myeloma IgE (Stanworth *et al.*, 1967) suggested that this pathological immunoglobulin possessed a strong affinity for those same sites in isologous tissue to which human IgE antibodies bound (but there was no evidence that it possessed any antigen-binding capacity). This was further suggested by the inability of the myeloma patient (ND) to become sensitized following local injection of serum from our regular horse dander-sensitive donor (PR). We went on, therefore (Stanworth *et al.*, 1968), to investigate the P–K inhibitory activity of proteolytic cleavage fragments of the myeloma IgE. These were derived from various regions of the molecule by papain and pepsin cleavage (as indicated in Fig. 3), being separated by gel-filtration on Sephadex (G-150) and chromatography on DEAE-Sephadex. It was encouraging to find that only the Fc fragment (at a dose

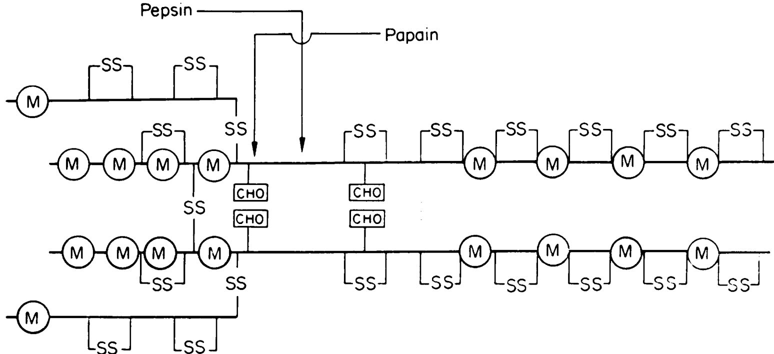

FIG. 3. Schematic representation of structure of myeloma IgE (reproduced from Bennich & Johansson, 1967).

of 5 μg) showed the inhibitory activity of the parent IgE molecule, in both a competitive inhibition system and when the inhibitor was introduced into sites in the normal recipient's skin 24 hr prior to transfer of the allergic serum. All fragments derived from the other end of the IgE molecule were non-inhibitory, as was a Fc-like fragment which was of smaller size and lacked some of the antigenic determinants of the whole Fc fragment.

We have since fully substantiated these observations (Stanworth *et al.*, 1969a, b), by performing PCA-inhibition tests in baboons. We have again employed a competitive-inhibition system, injecting varying amounts of IgE fragment mixed with diluted allergic serum (this time from a grass pollen-sensitive individual) containing 13 ng (i.e. in the 0·1 ml injection) of total IgE [as estimated by the radioimmunosorbent test (RIST)]. Twenty hours later the animals received Evan's Blue (10 ml of 0·5% solution) followed by subcutaneous injection of a small amount of allergen (e.g. 4–5 mg) into a site remote from the passive sensitization sites. Again we have found that only the whole Fc fragments are inhibitory, at dose levels of the order of 2 μg (Stanworth *et al.*, 1969b), and moreover some of these proved less inhibitory than others depending on their electrical charge. It was also interesting to note that a fragment rich in carbohydrate located in the Fc region of the molecule was relatively non-inhibitory.

It is tempting to speculate that the conformational integrity of the bulky Fc region of the IgE molecule is essential for tissue sensitization. We have recently shown, however (Stanworth *et al.*, 1969a), that it is possible to cleave several of the nine intra-chain S–S bridges within this region (by partial reduction with 0·1 M 2-mercaptoethanol, followed by alkylation) without influencing its PCA-inhibitory activity. This does not necessarily mean, of

course, that *antibody* IgE would remain unaffected by such treatment; and, indeed, there is evidence to suggest that structural alterations within the Fc region are responsibe for loss of cell-binding activity following heating at 56°C.

We are now in the process of looking at the inhibitory activity of myeloma IgE fragments obtained by other methods of proteolytic cleavage and by chemical cleavage (e.g. with cyanogen bromide), using *in vitro* passive leucocyte and chopped lung sensitization as well as the monkey PCA system. A C-terminal cyanogen bromide cleavage fragment did not appear to be inhibitory *in vitro*, suggesting that the tissue binding sites are not located at the ends of the ε-chains. It will be interesting to try to establish whether they are located within the *hinge region* of the IgE molecule, as recent studies by Utsumi (1969) have suggested that structures within this region of rabbit γG-globulins are implicated in their binding to guinea-pig skin. (This is consistent, of course, with my earlier remarks about the structural basis of the lack of heterologous tissue-binding shown by human IgG2 globulins, which are over endowed with inter-chain S–S bridges within their hinge region.)

Hence a meaningful pattern is beginning to emerge in that all three types of anaphylactic antibody (i.e. IgE, γ_1 and γ_2) appear to react with target cells through side-chains located within the Fc regions of their molecules. This, of course, is what might have been anticipated because their Fab regions are then free for subsequent combination with antigen (allergen).

MECHANISM OF INTERACTION BETWEEN CELL-BOUND ANTIBODIES AND ANTIGEN

This brings me to the final aspect which I mentioned in the introduction concerning the mechanism of the reaction between cell-bound antibody and specific antigen, and the manner in which this initiates the release of pharmacologically active substances from the target cells (or secondary cells).

In considering possible ways in which this can occur, it is worth recalling the early observations of Ishizaka (1963) and others, which showed that pre-formed soluble IgG antibody–antigen complexes of certain proportions (e.g. Ag_3Ab_2) and non-specifically aggregated IgG globulins (e.g. rabbit and human) were capable of producing increased capillary permeability reactions in heterologous guinea-pig skin immediately on injection. It seems, therefore, that a cell-reactive configuration can be produced by prior combination of sensitizing antibody and antigen in the appropriate ratio provided that this comprises cross-linked antibody molecules. Ishizaka & Ishizaka (1968) have since shown that human IgE globulin can be likewise rendered cell-reactive (as revealed by intradermal injection into humans), either by specific combination with allergen (ragweed) or as a result of non-specific aggregation with bis-diazotized benzidine. We have confirmed this by tests with monomeric and aggregated forms of myeloma IgE. As is shown in Fig. 4, only the latter produced an immediate blueing reaction in baboons (following intradermal injection of 250 μg of each).

It seems likely, therefore, that cross-linking of sensitizing antibody molecules, whether they be of the IgE or IgG type, results in exposure (or production) of cell-reactive sites. Henney & Stanworth (1966) obtained convincing evidence in support of this idea in studies on pre-formed soluble complexes comprising rabbit IgG antibody and various antigens (e.g. BSA, ferritin). By optical rotation and chemical measurement, and by use of immunological techniques including re-immunization into other animals of similar allotypic specificity to the antibody donors, we were able to demonstrate that antigen–antibody

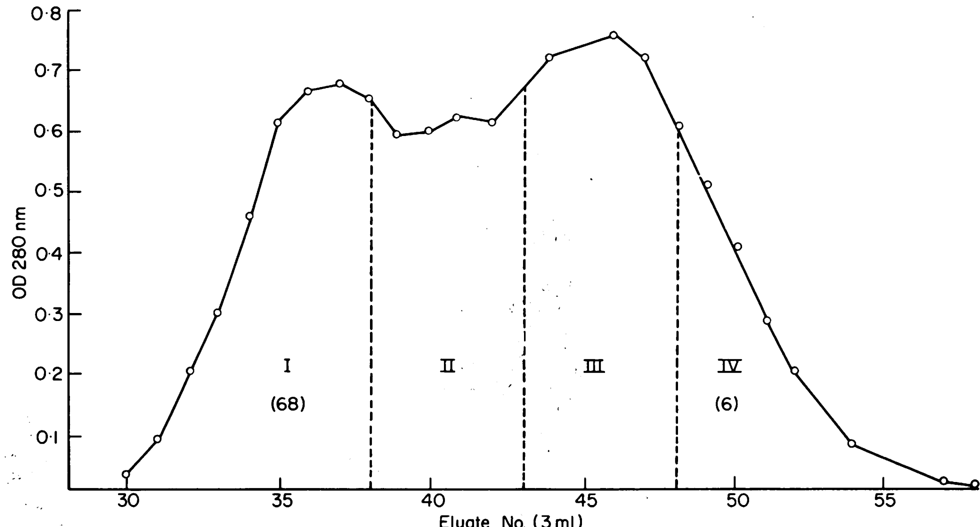

FIG. 4. Gel-filtration profile of a myeloma IgE (IgND) preparation containing aggregates, separated on a column (60 × 2 cm) of Sephadex G-200 using phosphate buffer (0·01 M, pH 7·5)–saline as eluant. The mean areas of blueing (in mm²), induced immediately on intradermal injection of 25 μg of the aggregate Fraction (I) and of the monomer Fraction (IV) into a baboon previously injected with 0·5% Evan's Blue (10 ml), are shown in parentheses.

combination in similar proportion to that capable of inducing an increase in capillary permeability could bring about the exposure of new antigenic determinants *within the Fc regions* of the IgG antibody molecules. This effect which is illustrated schematically in Fig. 5, can be looked upon as an allosteric transition induced within the Fc region of the molecule as a result of antigen combination at remote sites within the Fab regions. Further-

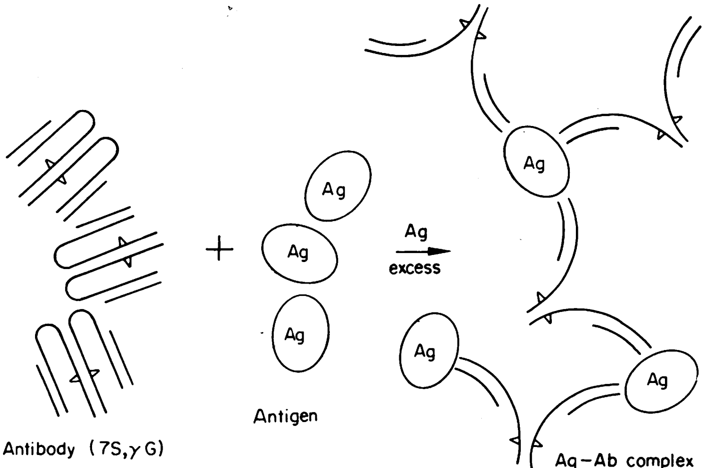

FIG. 5. Schematic representation of conformational changes induced in rabbit IgG antibody by combination with specific antigen (in moderate excess).

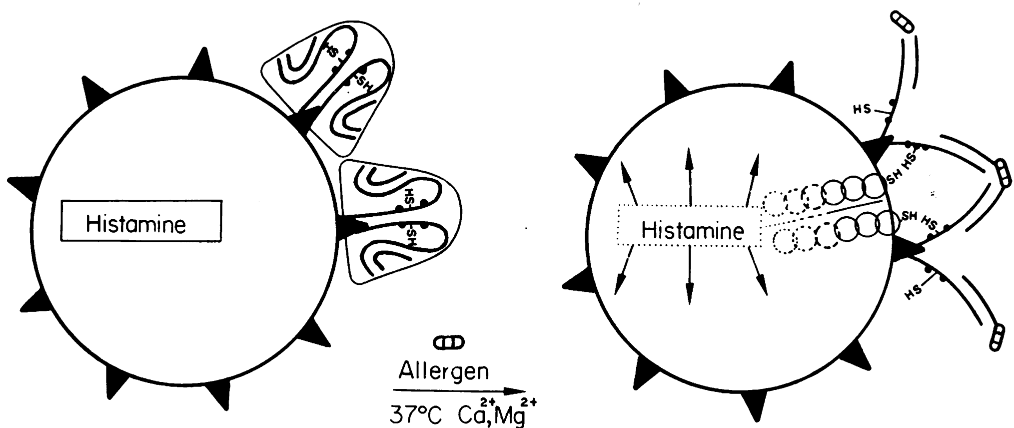

Fig. 6. Schematic representation of allosteric transition in cell-bound anaphylactic antibody following combination with specific antigen (i.e. allergen). (Reproduced from Stanworth, 1969, *Proc. Roy. Soc. Med.* **62**, 971.)

more Henney & Ishizaka (1968) have shown since that antigenically similar groups are revealed when rabbit IgG globulin is aggregated non-specifically.

I suggest, however, that a more subtle process occurs on reaction of cell-bound antibody (IgE or IgG) with its specific antigen (Ag), whereby an allosteric conformational change transmitted to that part of the antibody in intimate contact with the cell surface triggers off the chain of energy-dependent enzyme reactions which culminate in the release of histamine and other vasoactive amines (Stanworth, 1967, 1969). This effect is illustrated schematically

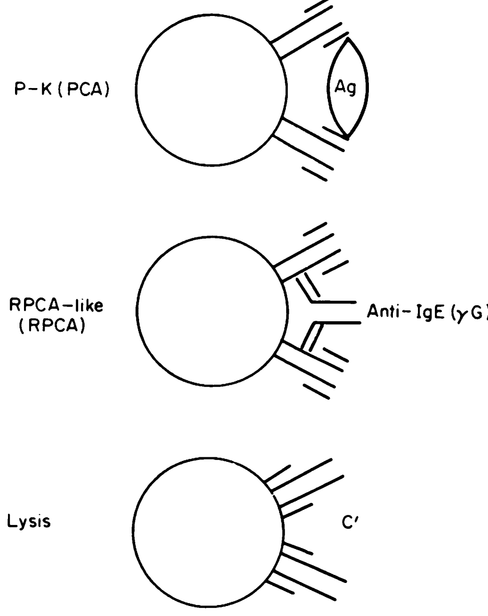

Fig. 7. Schematic representation of three major immunological methods of effecting the release of vasoactive amines.

in Fig. 6. Although exposure of an activating sulphydryl group is illustrated it is possible, of course, that some other type of active side-chain is involved or even a composite site comprising side chains from the adjacent antigen-linked antibody molecules. It might be thought that such a process would lead to disruption of the cell membrane as a result of a physical shearing effect. I maintain, however, that the effect transmitted to the cell surface would be relatively mild and selective in comparison with, say, the lytic process induced by combination with antisera raised against the cells themselves (indicated schematically in Fig. 7). In this case, antibody receptors within the Fab regions react directly with antigenic determinants which are an integral part of the cell membrane, and this appears to induce an interaction between the free Fc regions of adjacent antibody molecules leading to exposure of the complement fixing sites. There is no evidence that antigen-induced histamine release from IgE-sensitized cells is such a cytotoxic phenomenon; nor, surprisingly, is the release which can be induced in human and monkey skin by intradermal injection of anti-human IgE (Ishizaka *et al.*, 1969) which is also illustrated in Fig. 7. Lichtenstein & Levy (1969) have recently shown that this RPCA-like phenomenon, which Ishizaka *et al.* (1969) have shown requires the cross-linking of cell-bound IgE by antibody directed against its Fc determinants, involves *an active secretion of histamine* as do the antigen-induced reactions (also depicted in Fig. 7). It is important to recognize, however, that the RPCA reaction is an experimental system and for that reason might lack the finesse of the direct system, where the activation of a cellular pro-enzyme, say, might occur in the manner I have outlined as the result of a *two stage process* involving first cell-sensitization by antibody followed by provocation with antigen.

There are still many gaps in our knowledge of the immunochemical basis of the critical events occurring at cell surfaces during anaphylactic reactions. I feel confident, however, that these will be filled at an ever increasing rate during the next few years.

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