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The sensitization process can be separated from the process of antibody uptake. The amount of antibody retained by in vitro sensitized tissues is proportional to the concentration of antibody present in the bulk phase of the solution used for sensitization. Temperature has little effect on the amount of antibody retained by the tissue as a whole but does affect the degree of sensitization achieved. The velocity of sensitization is proportional to the bulk phase concentration of antibody used. Sensitization seems to be associated with the attachment of antibody molecules to very specific locations on sensitizable cells. This attachment process may involve the dissociation of antibody molecules from loose bonds with non-reactive cells and transfer to reactive cells. Experimental results have been presented which show that sensitization is related to the amount of precipitating antibody present in a mixture of antibody and homologous γ-globulin. In the case of guinea pig atrial tissue, antibody probably is attached to several sites on reactive, histamine-releasing, cells. The minimal number of sites on reactive cells has been estimated to be between ten and 200.

The presence of homologous γ-globulin in the sensitizing solution can reduce the degree of sensitization achieved. This inhibition process seems to result from the binding of non-specific molecules too close to antibody molecules so that subsequent reaction of the antibody with antigen is hindered sterically.

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LITERATURE REVIEW

Anaphylaxis, first described by Portier and Richet (188), is one of a group of phenomena which fall under the general heading of hypersensitivity. Induction of the hypersensitive state generally follows a primary or "sensitizing" exposure to an antigen. The antigen may be either a foreign protein or other large molecule with a certain amount of structural specificity, or a relatively simple structure which may be attached to the host's own proteins, somehow altering their relation to the host. After a certain length of time, the host produces antibodies, which are proteins that react specifically with the inducing antigenic material, and which may or may not appear in the circulation of the host. The second introduction of the antigen into the host, after the induction period, may cause one or more of the manifestations of the hypersensitive state. These manifestations may include circulatory collapse, respiratory distress, metabolic changes, tissue necroses, and, in some cases, death. Two main subdivisions have been made of the hypersensitive state. Originally, delayed hypersensitivity referred to the appearance of a reaction hours or days after the second dose of antigen. Immediate hypersensitivity referred to the elicitation of a response within seconds or minutes from the second dose of antigen. Anaphylaxis falls in the latter group. Now, the distinction between the two is made on the basis that immediate hypersensitivity can be produced in normal subjects by passive transfer of antibodies from sensitive animals, whereas the delayed hypersensitive state cannot.

The following discussion will be concerned primarily with the anaphylactic reaction and will not cover in any detail the mechanisms of delayed hypersensitivity, the mechanisms by which antigens stimulate the production of antibodies, the phenomenon of autosensitization, and serum sickness. We will be concerned specifically with the sensitization procedure, the subsequent release of pharmacologically active materials, and the appearance of the anaphylactic reaction.

General Features of Anaphylaxis and Analytical Techniques Used for Its Study.

The general features of anaphylaxis have been studied in complete animals (systemic anaphylaxis); the biochemical and biophysical steps in the reaction are being studied in isolated tissue systems (in vitro anaphylaxis).

a. Systemic anaphylaxis. The cause of death in anaphylactic shock was one of the first subjects to be investigated. It quickly became apparent that the cause of death varied from species to species. In the case of the dog, the target organ is the liver (227); in the case of the rabbit, the pulmonary arterioles and heart are the target organs (6, 7). Auer and Lewis (8) showed that in the guinea pig death was due to asphyxia caused by contraction of the bronchiolar smooth muscle. They showed that the reaction was independent of the vagus and was due to some direct action on the lungs.

Other important features of the anaphylactic reaction in the whole animal have been explored over the years. The necessity of a latent

period of a week or more between the initial injection and the effective challenging dose of the antigen was recognized in early studies (177, 199). The reaction was found to be highly specific, and it was found possible to sensitize a guinea pig to at least three different antigens simultaneously (52, 54, 199). Rosenau and Frost (199) were able to sensitize guinea pigs with the injection of one nanoliter (10^{-9} liter or 10^{-6} ml) of horse serum and, with guinea pigs sensitized with larger doses, were able to demonstrate a sensitivity lasting more than 160 days following a single injection. The failure of guinea pigs to respond anaphylactically under some conditions prompted the investigation of what was called anti-anaphylaxis. It was possible to render a guinea pig unresponsive by a series of daily injections of the antigen, and it was also found that if a guinea pig happened to survive an anaphylactic challenge, it remained unresponsive to the antigen for some time after the first challenge (3, 199, 232). Coulson, et al. (49, 50) have reported that the intensity of the anaphylactic reaction in guinea pigs depends on both the amount of antigen used to sensitize the animals and the amount used to challenge the animals. The amount of antigen required to produce a standard intensity of reaction was directly proportional to the amount of antigen used to sensitize the animals. Systemic anaphylaxis has been used as a test system to determine the immunologic responsiveness of the subject.

Studies of systemic anaphylaxis have been valuable in determining the ways in which whole animals react to challenge, and in determining ways to combat its effects. The limitations of this system in the study of the underlying mechanisms are obvious.

b. In vitro anaphylaxis. The next step in the development of study techniques was the observation that sensitivity could be transferred from a sensitized animal to a normal animal. This technique permits closer control of the amount of antibody involved in the production of sensitization. Kabat et al. (17, 105-108) used this technique to study the amounts and kinds of antibody required to produce systemic anaphylaxis in the guinea pig. However, the qualitative nature of the scoring procedure of the systemic anaphylactic reaction, even in the case of the somewhat more refined method of Pruzansky et al. (189-191), tends to mask the quantitative aspects of the anaphylactic reaction. It also is of limited usefulness in the investigation of the underlying mechanisms.

A further step in the study of anaphylaxis was the introduction of the use of isolated tissues. These could be prepared either from actively or passively sensitized animals. More recently, tissues have been passively sensitized in vitro. The last technique not only permits the experimenter to control the sensitization and challenge procedures, but also it permits a more quantitative measurement of intermediate responses. Many tissues have been used successfully for this purpose: the isolated gut, uterus, aorta, lung, tracheal rings, heart tissue, isolated rabbit platelets, and isolated mast cells. It is through these studies of the anaphylactic reaction of isolated tissues that most of our knowledge of the mechanism of anaphylaxis has been acquired.

c. Schultz-Dale reaction. W. H. Schultz (207) was one of the earliest to observe anaphylactic reactions in isolated tissues. While working at the Hygienic Laboratories (now the National Institutes of

Health) in Washington, D. C., he demonstrated that if loops of intestine taken from guinea pigs sensitized to horse serum were suspended in Ringer's solution, then, when horse serum was added to the bath, the loops would contract. However, his early work did not give a very clear demonstration of a specific reaction, and he interpreted his results as indicating that the isolated tissues were demonstrating only a slight increase in the normal sensitivity of the gut strip to horse serum. Dale (52) observed that an isolated uterus, prepared from a guinea pig which had survived an antitoxin test, contracted strongly when a normally innocuous concentration of horse serum was added to the organ bath. About the same time as Dale made his observation, Schultz published his preliminary report. Dale recalled the experiments of Smith (214) and decided to wait until a full report by Schultz was made before taking up the problem of anaphylaxis in isolated tissues. The somewhat equivocal results obtained by Schultz encouraged Dale to continue his researches, and he published a paper which became the foundation for much of the experimental work done in the field of anaphylaxis of isolated tissues.

The first analytical tool of quantitative studies of anaphylaxis was the anaphylactic contraction of isolated smooth muscle, which could be recorded by nonsubjective techniques. The excellent results obtained by Dale on the virgin guinea pig uterus, in contrast to the fair results of Schultz, made the uterus the experimental organ of choice for nearly 20 years. The work of Kendall and his associates (115) reestablished the value of the isolated guinea pig ileum as a test system. Although the guinea pig uterus is bi-cornate and one-half

may be used as control for the other half, the much greater amount of tissue available from the ileum has made the latter the system of choice in recent years. The anaphylactic contraction of smooth muscle in general has become known as the Schultz-Dale reaction.

d. Release of active material from shocked tissues. The observation that the anaphylactic reaction causes the release of pharmacologically active material has given investigators another test system with which they can estimate the anaphylactic reaction and parameters affecting it. Until recently, most assays of active material were performed on isolated smooth muscle which, under certain conditions, may be a very sensitive test system. For example, the isolated guinea pig ileum will contract in the presence of histamine concentrations of 1×10^{-8} M. It is possible to construct an isolated organ bath which would require the introduction of only 0.1 ml of unknown substance for assay purposes, which means that one could detect 10^{-12} moles of histamine with a fair degree of reliability. Chemical tests, which have the advantage of being specific and more quantitative, have been developed to the same degree of sensitivity only within the last few years. The fluorometric method of Shore et al. (211) now has become very competitive with bioassay for measurement of histamine, but the chemical assay still retains a certain amount of difficulty, and there is some delay in obtaining results, as compared with bioassay.

e. Cell reactions. The third analytical technique which has been used successfully is based on the observation that mast cells lose their metachromatically staining constituents during anaphylactic challenge.

This reaction has the advantage of providing numerical results directly. However, the problems associated with tissue preparation and observation of the relative proportions of degranulated cells in pieces of tissue make this technique unattractive. Isolated cell suspensions have been used, but the problems associated with this technique are not greatly different from those of the tissue cell population.

The Heart in Anaphylaxis.

a. Experimental animals. About the same time that Schultz published his work on the contraction of isolated guinea pig's ileum, Caesaris-Demel (38) showed that isolated hearts from sensitized rabbits and guinea pigs respond to anaphylactic challenge. He was careful to challenge with doses of antigen that were small enough to eliminate the effects of non-specific reactions. He noted changes in the rate and amplitude of contraction of his preparations. Auer and Robinson (9) reported electrocardiographic changes in a rabbit undergoing anaphylactic shock. They reported that the heart rate was slowed, and that often an ectopic beat developed. The anaphylactic reaction was not impaired by cutting the vagi. Later experimenters using isolated guinea pig and rabbit hearts (88, 128, 145, 231), isolated frog hearts (74, 118), and embryonic chick hearts (210), were not so careful as Caesaris-Demel, and it was difficult to distinguish between the effect of the anaphylactic challenge and the non-specific effects produced by the large doses of antigen used. Electrocardiographic studies of intact rabbits and guinea pigs have indicated that the reaction in the heart plays a secondary role to asphyxia in the guinea pig and to pulmonary arteriole constriction in the rabbit

(9, 47, 119). The electrocardiogram of isolated guinea pig hearts displayed prolonged PR intervals, changes in the QRS and T waves, and indicated the development of ectopic rhythms, and AV dissociation (4, 237). The coronary flow decreased and the contraction rate increased by 35 per cent. They noted that histamine gave parallel results and that atropine did not affect the heart rate changes but did reduce the change in flow. They also stated that the action of antigen was to depress the activity of rabbit atria. They studied the isolated hearts of cats sensitized to horse serum, and found that, during challenge, the coronary flow of the cat increased and the heart rate changed in no consistent pattern. Histamine gave qualitatively similar effects. Went (234, 235) found differences between the action of histamine and of the anaphylactic challenge on guinea pig hearts and attributed these differences to the release of choline or similar materials. The electrocardiograms of dogs show a tachycardia and depressed ST segment, but it is possible that these effects are due to the vascular collapse associated with the anaphylactic reaction in the dog, rather than to direct action on the heart (228).

b. Human cardiac anaphylactic effects. Siegal (212) has recently reviewed the role of heart reactions in human anaphylaxis. Electrocardiographic disturbances have been observed during several cases of severe anaphylactic reaction (21, 24, 70, 202). The reported disturbances include T wave alterations, AV dissociation, and atrial fibrillation. Although Castberg (40) felt that the changes observed resulted primarily from asphyxia, there appears to be evidence that the human heart can undergo an anaphylactic reaction.

c. Active materials released from shocked hearts. Went has suggested that choline is released from a shocked guinea pig heart (234, 235), but more recent studies have reaffirmed the parallel action of histamine and the release of histamine from challenged, sensitized tissues. Feigen et al. (66) demonstrated by pharmacologic and chemical tests that the isolated guinea pig heart released histamine during anaphylactic challenge. Acetylcholine hyperpolarizes atrial muscle cells, whereas histamine depolarizes. The cholinergic material observed by Went may have been released from damaged cells after the anaphylactic reaction. Mannaioni et al. (137-140) showed that the only substance which inhibited the anaphylactic reaction of the isolated guinea pig heart was a compound, dichloroisopropylarterenol, which also prevented the liberation of histamine during the anaphylactic challenge. The action of anaphylactic shock in the isolated atria has been studied, and the parallelism between the action of histamine and the anaphylactic reaction has been shown not only for the chronotoropic and inotropic behavior but also for the electrophysiological changes (66, 76, 117, 186). Quantitative studies of the anaphylactic reaction have been made (67) and have been enlarged upon and will be described in the later sections of this thesis. We will show that the isolated guinea pig heart is capable of releasing large amounts of histamine during anaphylactic challenge and that these tissues are useful in determining some of the conditions which affect the magnitude of histamine release.

d. Other effects on the heart. The mechanisms of the long-term effects of antigen-antibody reaction are beyond the scope of this

discussion, but it should be pointed out that pathologic changes not associated with the immediate effects of anaphylaxis have been noted in heart tissues (116, 133, 136, 208).

The Mechanisms of Anaphylaxis.

The combination of antigen and antibody on, in, or near certain cells starts a chain of reactions which culminate in the release of pharmacologically active materials, and it is the action of these materials on the smooth muscle and other tissues which produces the physiological symptoms of anaphylactic shock. We are concerned primarily with the reactions leading up to the release of pharmacologically active materials.

a. Early theories. As was pointed out, early investigators of the anaphylactic phenomenon attributed the shock to an increased susceptibility to the toxic nature of the antigen. However, it quickly was shown that materials which were innocuous to normal animals, even at relatively high concentrations, would produce anaphylactic shock in sensitized animals. The fact that sensitivity could be transferred to normal animals by the injection of the serum from sensitized animals led investigators to conclude that the anaphylactic reaction was associated with some property of the blood. For example, Richet (193) suggested that the reaction of the antigen and a material produced by the animal in response to the primary injection of antigen was to produce a poisonous product called "apotoxine" which in turn produced symptoms of anaphylactic shock. However, the work of Dale (52) and Caesaris-Demel (38) made it appear

that it was not necessary to have more than the antigen present in the solution incubating or perfusing isolated sensitized tissues. There arose two views on the nature of the anaphylactic reaction. The first was that the reaction of antigen and antibody in solution caused the formation of a material called anaphylatoxin which then produced the symptoms of anaphylactic shock; and the other, proposed by Dale, was that the cells of the tissues themselves were sensitized, and it was the action of the antigen on the sensitized cells that produced the symptoms. Both the humoral and cellular theories of anaphylaxis have undergone modification over the years, but neither theory has been proven conclusively. The eventual explanation of anaphylaxis probably will include elements of both theories.

b. Reactions similar to anaphylaxis. The anaphylactoid reaction, as it has become known, was first noted by Vaughan (225) in 1907. Somewhat later Friedberger (71) duplicated many of the symptoms of anaphylactic shock by incubating normal serum with an antigen-antibody precipitate. Other workers were able to treat normal serums with a wide variety of materials including agar, starch, and even barium sulfate (55). However, Dale and Kellaway (55) observed that isolated tissues and whole animals could be desensitized to the effects of anaphylatoxin and yet retain anaphylactic sensitivity, and vice versa. This effect has been noted repeatedly over the years for other tissues including the heart (76) and guinea pig mast cells (163).

c. Antigen-antibody complexes. Recently, a great deal of interest has developed in what may be an anaphylactoid phenomenon. Germuth and

McKinnon (72) showed that rabbit antisera treated with excess antigen produce the symptoms of anaphylactic shock in normal guinea pigs. Trapani et al. (221) were able to cause ileal strips to contract like a Schultz-Dale reaction using soluble antigen-antibody complexes. However, the reaction was not as rapid as the conventional Schultz-Dale. Ishizaka et al. (94-98) studied the biologic activity of soluble complexes and found that the best skin reaction was obtained with complexes made on the antigen-excess side of the equivalence zone. They reported that free antigen was not important in producing the effects of antigen-rich complexes, and that the complement fixation properties of the complex did not parallel its skin reactivity. Exogenous γ-globulin inhibited the skin activity of the complex. They observed small changes in the optical activity of the γ-globulin after it had combined with antigen, and suggested that the process of combination produced a slight distortion of the protein structure. This distortion of structure could be transmitted to the cell upon which the γ-globulin was fixed and act as a stimulus for the subsequent steps of the anaphylactic response. Heat-aggregated γ-globulins were found to fix complement in the presence of divalent cations and to cause skin reactions (46, 103, 217). In Friedberger's experiments (71) it was demonstrated that there was a need for the complement fraction of the serum in order to produce the anaphylactoid effects. The experiments with soluble complexes also were performed with antisera. Purified antigen-antibody mixtures, made in the absence of other serum factors, have been much less effective than mixtures prepared from antisera (172, 201). Soluble complexes are able to produce some of the effects of anaphylaxis. This

observation has suggested a simple immuno-chemical mechanism for the action of antigen on sensitized tissue. However, the differences between the effects of complexes and anaphylactic shock, and the unclear role of serum factors in the activity of complexes have made investigators look beyond this simple mechanism. It should be pointed out that circulating immune aggregates do seem to be important in the elicitation of the Arthus phenomenon.

d. Anaphylatoxin and histamine. Treatment of serum (usually from rats) with certain agents produces a material called anaphylatoxin. If anaphylatoxin is injected into a guinea pig, it soon develops the symptoms of anaphylactic shock. The parallel action of anaphylatoxins and histamine on intact animals and isolated tissues is evidence for the suggestion that anaphylatoxins act as histamine releasers. Osler et al. (176) reached this conclusion and showed that the presence of immune aggregates in fresh sera produced the fixation of the C'_3 component of complement. Halpern (79) showed that long-chain molecules, such as dextran and polyvinylpyrrolidine, were also capable of releasing histamine. He found that optimum release was obtained when the molecular weight of the dextran was about 100,000. It is interesting to compare this value with the molecular weight of immune aggregates of Ag_2Ab composition, which, in the case of ovalbumin-antiovalbumin, would be about 240,000, or of Ag_3Ab_2 composition, which would weigh about 440,000. The mechanism for the release of histamine by long-chain molecules or aggregates has not been found. Histamine is released during anaphylactic shock and by anaphylatoxin. Rat and guinea pig mast cells are degranulated (157, 163),

but there is evidence that the two reactions have somewhat different pathways. It is possible to desensitize tissues to anaphylatoxin and yet still have them retain their reactivity to anaphylactic shock (55, 163). Shock by anaphylatoxin is not so dependent on calcium or temperature as anaphylactic shock. Histamine release may not be the only consequence of anaphylatoxin challenge. Scheiffarth (205) was able to deplete a large fraction of the histamine of guinea pigs with stilbamadine, but this treatment did not prevent the induction of anaphylactoid shock. The exact nature of anaphylatoxin is not known, but it can be produced regularly, and certain of the important factors in its formation have been characterized (146). It appears to be a histamine releaser, but its formation does not seem to be necessary for or associated with the production of anaphylaxis in isolated tissues.

e. Histamine releasers. Some materials mimic the effects of anaphylaxis by causing the release of histamine from tissues. Studies of these releasing compounds and of materials which inhibit the release by these compounds has given us a great deal of knowledge about the possible mechanism of histamine release. Some rather simple compounds were found to release histamine and, as most of them were basic materials, it was assumed that the release mechanism was essentially a displacement of the histamine base from its ionic attachment to acidic material, such as heparin (78, 79, 184, 185). However, more recent investigations have shown that this is not necessarily the case (51). The observation by Högberg and Uvnäs (88) that compound 48/80 had a sharply defined pH optimum and required calcium for its action suggested to them that this

releaser worked by means of enzymes, rather than displacement. Surface active agents, such as octylamine or decylamine, probably do not act through an enzyme system, but disrupt mast cells by their direct effect on the cell membrane (88, 158). Although the histamine released by compound 48/80 and by antigen-antibody reactions may have a common path, there are distinct differences between the two. Whereas iodoacetate inhibits anaphylactic release of histamine, it seems to potentiate the action of 48/80 (152, 153). Mongar and Schild (147) showed that the degree of histamine release caused by treatment of guinea pig lung tissue with 48/80 was very sensitive to its concentration in contrast with the effect of antigen concentration. A ten-fold increase in 48/80 concentration produced the same increase in histamine release as a four decade increase in antigen concentration. Halpern et al (83) found that hypothermia increased the release by 48/80. Another histamine releaser is d-tubocurarine which has been shown to release histamine from the rat diaphragm (197) and other tissues (147). Anaphylatoxin has been mentioned earlier as a histamine releaser.

The Mechanism of Passive Sensitization.

Although Dale (52) presented some evidence to show that passive sensitization could be accomplished in vitro, it was not until Hartley (84) sensitized uteri of normal guinea pigs by soaking them overnight in cold antibody solution that passive sensitization of isolated tissues was demonstrated. Since that time, passive sensitization of isolated tissues has become routine practice. Among the tissues used for studies of the mechanism of sensitization are chopped guinea pig lung, guinea pig ileum, heart, and the skin of the intact animal.

a. The role of antibody-type in sensitization. Sensitization of normal tissues can be accomplished by soaking them in solutions of either antisera or the immune globulin fraction of the sera. The source of the antibody is important in determining whether or not a sensitization occurs. Ishizaka et al. (101) found that ileal strips taken from guinea pigs injected with horse diphtheria antitoxin would not demonstrate a Schultz-Dale reaction when challenged with diphtheria antigen. Humphrey and Mota (91) were able to induce systemic anaphylaxis by sensitizing guinea pigs with simian antiovalbumin and canine anti-egg white but were unable to produce the reaction with antisera made in the goat, fowl, rat, or horse. They found that they were unable to obtain a reversed passive cutaneous reaction unless the antigen were γ-globulin, but horse and goat γ-globulin also failed to produce the reversed reaction. They tagged the antibody with radioactive iodine and found that all the antibodies became adsorbed to the tissues and could bind complement. Ovary (179) found similar results but was able to produce a reversed passive cutaneous anaphylaxis (PCA) reaction with horse anti-human γ-globulin as the antibody and human γ-globulin as the antigen.

The sensitization phenomenon has been separated into two steps: one, adsorption of the antibody or, perhaps more generally, uptake of the antibody by the tissues, and two, a process called fixation. The ability of an antibody to fix or sensitize a tissue depends not only on its source but also on certain structural features of the antibody molecules. The work of Ishizaka et al. (99) indicates the need of intact disulfide linkages in the antibody molecules for sensitization to occur. Ovary et al. (178, 181, 182) have studied the effects of enzymatic

digestion of antibody and found that the fragments associated with the combining sites inhibited neither the PCA nor the reversed PCA, while the non-combining site fragment (Porter fraction III) would do both. Further digestion of the antibody with papain impaired the production of the reversed PCA.

The heterogeneity of γ-globulins and antibodies often has been demonstrated (62). Some investigators have suggested that there is a particular kind or type of antibody which is responsible for sensitization. Associated with this question is the problem of the relation between precipitating antibodies and sensitizing antibodies. Although the sensitizing ability of antisera generally parallels the precipitin content, it has been possible to sensitize guinea pigs with the non-precipitating fraction of rabbit antibody (105). Boyden and Sorkin (34, 35) treated spleen cells with antibody and then with radioactive antigen and found that the amount of radioactive antigen taken up by cells did not parallel the amount precipitated from the whole sera. However, they did not study the ability of their "cytophylic" antibody to sensitize tissues. When Brocklehurst et al (37) screened radioactive antibody by injecting it into animals and then recovered the antibody by precipitation of the globulin fraction of the "screened" sera, they were unable to demonstrate any loss of sensitizing ability. Swineford and Samsell (219, 220) were unable to separate the sensitizing fraction from the precipitating fraction. Benacerraf et al. (18, 26, 180) found evidence for three types of antibody produced by the guinea pig. One of these was associated with complement fixation and hemolysis, another was connected with the production of systemic and passive cutaneous anaphylaxis, and the third with

complement fixation only. Each type of antibody appeared at different times in the course of active sensitization. The skin-sensitizing and precipitating activity of rabbit antisera have different electrophoretic mobilities (1, 94) and appear at different times in the course of immunization (224). Heimlich et al. (87) were unable to find a correlation between heavy (19S) and light (7S) antibodies with respect to their ability to produce skin reactions.

The method of producing the antibody seems to be important in the production of sensitizing or precipitating antibodies. Bauer et al. (16) reported that 19S γ-globulin appeared before 7S γ-globulin in rabbits immunized with alum precipitated antigen. Similar findings have been reported by Benedict et al. (19) for chicken antibody production. Benacerraf et al. (18) reported that antigen incorporated with adjuvant induced in guinea pigs the formation of γ-globulin with low electrophoretic mobility, whereas fast migrating γ-globulin was formed when no adjuvant was used. Baserga and Bergamini (15) gave guinea pigs repeated injections of antigen, which caused them to lose their anaphylactic sensitivity; however, the serum had a high precipitin titre. When they injected the antigen mixed with Freund's adjuvant, they found that the precipitin content was high and the guinea pigs retained their anaphylactic sensitivity. The sort of adjuvant used is also important. Munoz (170) reported that rats injected with antigen in Freund's adjuvant produced antibody with high precipitin content, but the sensitized rats were less susceptible to anaphylaxis than rats sensitized with antigen and *B. pertussis* organisms. Mota (161, 162) has reported similar findings and suggests that the rat produces "mast cell lytic" antibodies. The

formation of antibodies by the reticulo-endothelial system seems to depend on the mode of introduction of the antigen; it would seem reasonable that the physical properties of each type of γ-globulin produced are important in determining their role in the precipitin reaction, in the induction of sensitization, and the production of anaphylactic sensitization. Bloch et al. (26) have shown this to be the case for guinea pig γ-globulins.

b. Antibody uptake and sensitization. Several investigators have used radioactively tagged antibody to measure the relation between the induction of sensitization and the amount of antibody taken by the tissues. Ishizaka et al. (100, 102) passively sensitized whole guinea pigs and 24 hours later removed the ilea for Schultz-Dale reactions. They found that there existed an hyperbolic relation between the log of the antibody concentration found on the tissues and the log of the antigen concentration used to evoke a standard Schultz-Dale reaction. Feigen et al. (65) passively sensitized guinea pig ilea in vitro and concluded that the amount of antibody bound to the tissues was related by a simple Langmuirian isotherm to the bulk phase concentration used to sensitize the tissue, and that the degree of sensitization was related to the amount of antibody bound. Brocklehurst et al. (37) used radioactive antibody to study the amount of uptake and degree of sensitization in chopped guinea pig lung, and found that the uptake of antibody by the tissue continued long after sensitization was complete, especially when the tissues were incubated in high concentrations of antibody. The amount of antibody required to produce sensitization is quite small. Binaghi et al. (22) were able to

sensitize guinea pig ileal strips with solutions containing as little as 2×10^{-9} g of antibody nitrogen/ml. Kritzman and McCarthy (120) substantially suppressed the amount of circulating γ-globulin in guinea pigs but were unable to reduce their sensitivity to anaphylactic shock. In the experimental section of this thesis, evidence is presented that as few as 10 molecules of antibody are required to sensitize guinea pig mast cells.

The degree of sensitization of tissues depends on the amount of antibody in the tissues, but the magnitude of the anaphylactic reaction depends on the temperature, pH, etc., as well as how much antibody is present. The presence of so much non-reactive adsorbing surface in tissues tends to mask the events which occur in the reactive tissues.

c. Effect of temperature. The effect of temperature on the sensitization of isolated tissues is to change the rate of sensitization (63-65, 82). Temperature has very little effect on the amount of uptake of antibody; Feigen et al. (64) showed that there was no difference in the amount of uptake of antibody by isolated guinea pig ileal strips when they were incubated at either 37 C or 20 C. Brocklehurst et al. (37) found that reduction of incubation temperature to 0 C reduced the amount of uptake by only a factor of two. Comparable degrees of sensitization were obtained when lung tissue was incubated for four hours at 37 C or in the same concentration of antibody for 50 hours at 4 C (155). It has been found that the temperature history of the tissue, after its exposure to antibody, is very important in the degree of sensitization achieved (63). The activation energy of the sensitization process,

apart from antibody uptake, has been found to be quite high. Nielsen and Feigen (173) estimated it to be 18 kcal for guinea pig ilea, while Mongar and Schild (150) found that the Q_{10} of sensitization was nearly 12. Our studies to be described in the experimental section of this thesis, generally confirm these results for heart tissues.

d. Inhibition by serum proteins. The presence of non-antibody proteins affects the sensitization process. One of the earliest observations was the fact that guinea pigs which were repeatedly injected with small doses of antigen became clearly immune or anti-anaphylactic. This phenomenon was attributed to the presence in the circulation of excess antibody which reacted with the antigen before it had a chance to reach tissue-bound antibody (15, 93, 219, 220). Dale (52) showed that the tissues taken from immune animals displayed a typical Schultz-Dale reaction when challenged in an isolated organ bath. Anti-anaphylaxis seems to be dependent on the presence of very large amounts of circulating antibody, for Morris (156) found that the amount of antigen required to kill passively sensitized guinea pigs decreased as the sensitizing dose of rabbit antibody was increased moderately.

Ovary et al. (180) separated guinea pig antiovalbumin electrophoretically into a fast and a slow component. The fast component seemed to be involved with production of systemic anaphylaxis, and a mixture of fast and slow in a ratio of 1 to 100 completely inhibited the elicitation of systemic anaphylaxis. Systemic anaphylaxis in guinea pigs and mice could be inhibited if large amounts of human γ-globulin or rabbit serum were given before challenge, but neither horse nor bovine γ-globulins were able to protect animals against systemic anaphylaxis (80, 81).

Fisher and Cooke (69) found that normal rabbit serum inhibited the PCA and, on further investigation, found that the γ-globulin portion of the serum was responsible for the inhibition. Biozzi et al. (25) tested the effectiveness of mixtures of antibody and normal serum proteins to produce PCA's. They found that the "competition ratio", which they defined as the threshold antibody dilution divided by the threshold antibody plus serum protein dilution for equal size reactions, varied linearly with the γ-globulin concentration. Ishizaka and Campbell (95) also noted that non-specific γ-globulin can inhibit the skin activity of soluble complexes. Ovary and Karush (178, 181), using papain-digested rabbit antibody, found that fragments containing the combining site would neither inhibit PCA or cause PCA. Porter fraction III inhibited the PCA and would cause a reverse PCA when challenged with antibodies against rabbit γ-globulin. Ishizaka et al. (101) found that treating rabbit antibody with both mercaptoethanol and iodoacetate reduced the amount of precipitate formed in the antigen excess zone. When the antibody was treated with either mercaptoethanol or iodoacetate alone, there was no effect on the reversed PCA. When both were used to treat the antibody, the reversed PCA reaction was completely inhibited. The treatment did not seem to affect the complement fixing properties of the antibody. Liacopoulos et al. (132) found that bovine γ-globulin and human γ-globulin and human serum albumin, but not bovine serum albumin, inhibited the delayed skin reaction to picrylated guinea pig albumin. They believe that the inhibition was due to competition for reaction sites and not to the suppression of antibody formation.

The inhibition of passive sensitization of isolated tissues by normal sera or γ-globulins also occurs. If tissues are perfused with physiological saline prior to the sensitization procedure, the amount of sensitization produced is increased (130, 151, 155). In their studies with radioactive antibody, Brocklehurst et al. (37) found that the presence of a high concentration of exogenous γ-globulin increased the rate of elution of antibody from the tissues. If the tissues were allowed to remain in contact with the antibody for long periods of time, the presence of non-antibody γ-globulin did not reduce the degree of sensitization as much (37, 155). The velocity of sensitization of guinea pig lung tissue was reduced when the antibody was mixed with normal guinea pig or rabbit sera, or human γ-globulin (155). Binaghi et al. (23) found that the velocity of sensitization of isolated guinea pig ilea varied with the square root of the concentration of γ-globulin when the ratio of antibody to γ-globulin was maintained constant. The velocity varied directly with the concentration of antibody when the γ-globulin concentration was constant, but the ratio of antibody to total γ-globulin was varied (23, 82). The inhibition of sensitization was not due to the α- or β-globulin or to the albumin component of the sera (82). Based on their studies with sensitized guinea pig lung tissue, Mongar and Schild (155) reported that bovine γ-globulin (BGG) did not inhibit passive sensitization by rabbit antibody. On the other hand, Neu et al. (171) found that BGG did inhibit the passive sensitization of guinea pig ileal strips with rabbit antibody. Although the work of Binaghi et al. (23) suggested to them that γ-globulins react in solution to form dimers, and that it was the existence of non-antibody γ-globulin coupled to antibody γ-globulin

that prevented the mixture from reacting with antigen, other workers including ourselves believe that the inhibition phenomenon is a competition for reaction sites between antibody and non-antibody molecules.

e. Other factors. The sensitization process does not seem to be affected by metabolic inhibitors such as cyanide, azide, phenol, DNP, calcium lack (151), or fluoride (155). Binaghi et al. (22) found they could increase the rate of sensitization of guinea pig ilea by using antibody in an isotonic glucose solution buffered with a small amount of sodium bicarbonate. They were able to produce sensitization of tissues with 2 nanograms of antibody nitrogen/ml, whereas raising the ionic strength to 0.02 increased the sensitization time by a factor of three.

The Effects of Antigen on Sensitized Animals and Tissues.

The introduction of antigen into a suitably sensitized animal causes the contraction of smooth muscle and the release of pharmacologically active materials. Many investigators have studied the effects of ionic environment, temperature, pH, and metabolic inhibitors on the anaphylactic reaction in order to obtain some information about the intermediate steps involved during anaphylactic shock.

a. Location of the antigen-antibody reaction. The location of the antigen-antibody reaction which starts the chain of events leading to the anaphylactic reaction generally is considered to be the cell surface, although there is no conclusive evidence to support this assumption. It is possible that the reaction takes place in solution near the surface of the tissues starting with the formation of an antigen-antibody

complex, or, as has been suggested by some investigators, the initial step could take place within the tissues. Humphrey and Mota (92) were unable to show by the fluorescent antibody technique that antibody adhered preferentially to any particular tissue in the guinea pig mesentery. Van den Berg et al. (20) used fluorescent antigen to demonstrate that during a PCA reaction antigen could be found in the vessel walls, within the lumen of the vessel, and free in the tissues; they found granulocytes with antigen in the cytoplasm within five minutes after challenge. Electron microscope studies of ferritin-conjugated antibodies to ascites tumor cells showed that without complement the antibody stayed on the membrane surface; when complement was added, the cells expanded and ferritin was found within the cytoplasm (61). The presence of antigen-antibody complexes at the site of the Arthus reaction has been demonstrated (20, 169), and the complexes seem to be involved directly in the production of the reaction. Godlowski et al. (73) concluded from their studies of the effects of antigen-antibody complexes that the complex entered the cell body before the anaphylactic reaction took place. They showed that fluorescence-tagged complexes entered uterine cells of sensitized and non-sensitized guinea pigs, but only the uterine horns of sensitized animals gave Schultz-Dale reactions. Their evidence for an intracellular reaction is not conclusive, as the guinea pig anti-HGG could have reacted with the precipitates at the cell surface, as well as inside.

b. Release of active material. Early investigators of anaphylaxis noted the parallel action of histamine and anaphylactic shock on the guinea pig (53, 56), but it was not until 1932 that the actual release

of histamine was demonstrated by Bartosch et al. (14). Since that time, many other active materials have been found to be released subsequent to the anaphylactic reaction. Kellaway and Trethewie (111) found a smooth-muscle contracting material which they called Slow-Reacting Substance. More recently, a different kind of Slow-Reacting Substance (SRS-A) has been found to be released during the anaphylactic reaction. This material is lipid-soluble in contrast to the peptide nature of the Slow-Reacting Substance of Kellaway and Trethewie. The lipid-soluble material has been found to be released from heart, liver, and lung tissues (30, 36, 43, 44). The release of SRS-A parallels the release of histamine but is delayed somewhat in time. Chakravarty and Uvnäs (44) found that it was possible to cause histamine release without causing the appearance of SRS, but they were unable to release SRS-A without a simultaneous release of histamine. Campbell and Nicoll (39) found another material released from guinea pig lung tissues during anaphylactic shock. This material contracted the rat uterus which is insensitive to histamine. They suggested that the material was choline or acetylcholine, but later investigators have concluded that the active substance was serotonin (5-hydroxytryptamine). Boreus and Westerholm (31) found that no serotonin was released from rat gut, heart, or spleen, and that very little was released from guinea pig tissue. They concluded that serotonin made no contribution to the Schultz-Dale reaction. Waalkes and Coburn (230) found that the release of serotonin from rabbit platelets undergoing anaphylactic reaction paralleled the time course of histamine release. Went and Lissak (235) found what they considered to be acetylcholine released from challenged guinea pig heart, but we and others have failed

to confirm this finding. (See the section on The Heart in Anaphylaxis).

The appearance of active agents as a consequence of an anaphylactic reaction accounts for many of the observed manifestations of anaphylactic shock. There are some conditions in which the release of active materials is very small, as in the case of the Schultz-Dale reaction. Dale (55) has suggested that the release of active material by sensitized cells in the immediate vicinity of smooth muscle cells causes the local concentration of the active material to be very high, although the total amount of substance might be very low. This suggestion accounts for the observation that antihistamines are not entirely effective in abolishing Schultz-Dale reactions, but block the action of added histamine at concentrations which would be expected to cause substantial smooth-muscle contraction. Others have suggested that smooth muscle cells themselves become sensitized, and that the action of the antigen upon the antibody at the cell membrane can cause the smooth muscle to contract (172). These points have not been resolved.

c. Mast cell reactions. The source of histamine has been attributed to the presence of histamine in mast cells which are found in varying numbers in the connective tissue of almost every organ of the body (195, 196). Mast cells were shown to contain heparin by Jaques and Waters in 1941 (104), and more recently, the existence of histamine in mast cells has been shown by Riley (195). Riley and West (196) found that the mast cell population paralleled the histamine content of a variety of tissues. The function of mast cells is not understood, but Riley (194) has suggested that the release of histamine from degranulated

mast cells causes local edema which attracts phagocytes, and that the heparin which is released from the granules is a chemotactic agent for the phagocytes. Smith (213) observed the response of mast cells to toluidine blue. He noticed that initially the granules disappeared and then reappeared stained purple. He suggested that histamine was liberated from the heparin allowing the dye to stain it. Histamine is basic and heparin is acidic, and it generally is believed that the two are bound together in the intact cell by electrostatic or other relatively weak forces. The degranulation of mast cells has been observed when they have been treated with not only toluidine blue, but water (192, 195) and known histamine releasers such as 48/80, octylamine, and decylamine (27, 79, 88, 158, 167), antihistamines (165), antigen-antibody reactions (5, 28, 88, 89, 92, 158, 159, 161, 163, 165), anaphylatoxin (157, 163), and certain enzymes (27, 112, 223). If the mast cells are broken mechanically, the histamine-containing particles remain intact. Water will release the histamine, as will 48/80 and octylamine, but antigen-antibody reactions will not (77, 149). These results suggest that the antigen-antibody reaction takes place apart from the granules.

There are differences among the mast cell reactions of various species. Peritoneal exudates from guinea pigs are poor in mast cells, but many mast cells are found in the exudates of rats and hamsters (183). Boreus (27) compared mast cell reactions in the rat, hamster, and guinea pig; he found that the mast cells of the first two were disrupted by 48/80, lethicinase-A, and decylamine, while guinea pig mast cells were not affected by 48/80. Guinea pig mast cells also seemed to be more resistant to disruption by distilled water. Ninhydrin and ethylmaleimide

inhibit mast cell degranulation in rats and hamsters but not in guinea pigs. Humphrey et al. (89) have caused rat mast cells to disrupt by the application of rabbit anti-rat γ-globulin, but they were unable to disrupt mast cells from rats actively sensitized to HGG or hemocyanin.

d. Reactions of other cell types. Other cells have been shown to participate in the anaphylactic reaction. Shelley and Juhlin (209) showed that basophils taken from allergic patients would degranulate when exposed to antigen. Rabbit platelets release serotonin (90, 230). Hayashi (85) described a group of cells he called monocytes which showed morphologic changes during treatment with antigen.

e. The role of antigen concentration. The importance of antigen concentration on the degree of reaction was noticed by the early workers. Small doses of antigen would produce sub-lethal reactions in sensitized animals and submaximal reactions in isolated sensitized tissues. The response increases monotonically with increasing antigen concentration until a maximum is reached; thereafter as antigen concentration is increased, the reaction may be reduced. The increasing response with increasing antigen has been shown in the case of the gut (131, 171, 174), in the isolated heart tissues (this thesis), in studies on guinea pig mast cells (28, 92), and in the PCA (68, 201). In the case where the dose of antigen is very small, it is possible to produce a subsequent reaction by additional challenges with antigen (68, 129, 173, 200, and this thesis). Liacopoulos et al. (131) found that high antigen concentration inhibited the Schultz-Dale reaction. By sensitizing the tissues to two different antigens, they showed that the inhibition

was not due to a lack of reactivity of the tissue. The amount of antigen required to inhibit the reaction increased as the amount of antibody used to sensitize the tissues was increased. This also was found in the case of the lung by Brocklehurst et al. (37). It has been suggested by Liacopoulos et al. (131) that both low concentrations and very high concentrations react with antibody in the tissue to produce combinations that are unsuitable for the production of anaphylaxis. They found that, if sensitized tissues were allowed to soak in 0.016 μg antigen N/ml for various lengths of time, the tissues became desensitized to a subsequent large dose of antigen. The degree of desensitization was proportional to the duration of the pre-challenge exposure.

f. Temperature effects. The temperature at which challenge is performed has a pronounced effect on the degree of anaphylactic reaction. Schild (186) observed that when the challenge was made at 17 C, the guinea pig aorta released 15 per cent of the amount released at 37 C. In a later study, Mongar and Schild (150) found essentially no release from guinea pig lung below 20 C and above 45 C. The maximum anaphylactic release occurred around 40 C. At low temperatures reversible changes seem to take place, but at 45 C and above the changes appear to be irreversible (88). When tissues were challenged at 0 C, no release occurred but the tissues remained sensitized, whereas if they were challenged at 20 C, only a small amount of release occurred but the tissues became desensitized. These findings were confirmed by Chakravarty (42). The Q_{10} found by Mongar and Schild (152) was approximately 12. Our studies to be described below also indicate a very high temperature coefficient for heart tissues.

g. Ionic environment. The requirement for calcium ion also has been observed (42, 88, 89, 149). However, magnesium ion or potassium ion did not seem to be required. Changes in the ionic strength did not modify the reaction of rat mast cells with antigen (89), but Austen and Brocklehurst (12) found that in chopped guinea pig lung hypotonic solutions increased whereas hypertonic solutions reduced the amount of histamine released. This suggests that mast cells might be more fragile in hypotonic solution and thus more susceptible to challenge.

h. The effect of pH. The anaphylactic reaction has a broad reaction zone with respect to pH, being maximal at pH 7.8 and falling to about zero at pH 5 and 9.5 (42, 148, 150). There is a certain amount of interaction between the optimal calcium concentration and pH (148); the effect of reducing the pH can be offset partially by increasing the calcium concentration.

i. Metabolic inhibitors. The lack of oxygen or glucose has been found to inhibit partially the release of histamine from rat mast cells and from rat and guinea pig lung tissue (41, 42, 152, 153, 223). The absence of both is required for complete inhibition. Diamant and Uvnäs (60) suggest that the failure of some workers (88) to show that oxygen lack inhibits histamine release may have been due to the presence of glucose in the incubation solution. From his studies, Diamant (59) concluded that the glucose-requiring step was after the antigen-antibody reaction.

Inhibitors of various metabolic processes and various enzyme substrates have been tested for their effects on the anaphylactic reaction.

Their effects have been reviewed in detail by Mongar and Schild (146). Inhibitors of oxidative phosphorylation inhibit histamine release (58, 168, 223). Cyanide inhibits histamine release from guinea pig lung, but carbon monoxide does not (12). Observation of the inhibition of histamine release by iodoacetate and other sulfhydryl blocking agents has suggested to investigators that intact sulfhydryl groups are required for the anaphylactic reaction (41, 42, 152, 153). Pyridine and diphosphopyridine nucleotidase inhibitors also inhibit mast cell damage by anaphylactic reaction (166). Esterase inhibitors reduce the anaphylactic release of histamine from chopped guinea pig lung (13). Substrates of trypsin, leucine-aminopeptidase, and carboxypeptidase failed to inhibit the anaphylactic release of histamine from chopped guinea pig lung (13). Substrates of chymotrypsin were found to inhibit the release (10). Some monobasic fatty acids were found to inhibit histamine release, while certain dibasic aliphatic acids enhanced the anaphylactic release of histamine (11). Keller and Beeger (113) also found evidence for a chymotrypsin-like enzyme being involved in the anaphylactic reaction.

j. The importance of complement. The role of complement in the anaphylactic reaction has not been resolved. The early work of Dale (52) showed that it was not necessary to have complement in the challenge solution of isolated uteri. However, it has been suggested that there is a tissue complement or that the complement factors were not removed completely by a perfusion procedure. The presence of fresh serum does potentiate the Schultz-Dale reaction and mast cell disruption (5, 89, 123, 124). The toxic effects of antigen-antibody complexes seem to depend on

the presence of serum factors; complexes made with purified antibody and antigen fail to produce the effects observed with the complexes made from antigen and antisera (74, 172). Ishizaka et al. (102) suggested that complement was not involved directly in passive in vitro reactions because complement fixation is inhibited by high antigen concentrations. The Schultz-Dale reaction was not inhibited over the range of concentrations they studied. Humphrey and Mota (91) found the ability of antibodies to fix complement did not parallel their ability to sensitize guinea pig tissues. Bloch et al. (26) found that the slow component of guinea pig antibody fixed complement and was involved in the Arthus reaction. The fast component did not fix complement and was associated with PCA and systemic anaphylaxis. Finally, Austen and Brocklehurst (10) tested a variety of enzyme substrates and inhibitors on the release of histamine from chopped guinea pig lung and found at least six differences between the anaphylactic reaction and immune hemolysis. However, the evidence is not conclusive and it may be that certain reactions can mimic anaphylaxis in the presence of complement, whereas other reactions may not need complement. Also it may be possible that the serum factor which enhances the reaction is not complement.

k. The role of histaminase. Some investigators have suggested that the histamine destroying system, generally referred to as histaminase, is released simultaneously with histamine during anaphylactic shock, and that this enzyme mitigates the effects of histamine during shock. Lindell and Westling (134) measured the histaminase activity of different tissues in the guinea pig and found that activities ranged from 0.2 μg per g of tissue per hour for striated muscle up to 22 μg per g per hour

for the liver. The lung, which is the shock organ in the guinea pig, had an activity comparable to striated muscle, and there is a good deal of doubt as to whether lung histaminase *per se* would reduce the severity of the anaphylactic reaction. Westling (236) subjected unanesthetized guinea pigs to an injection of histamine and measured the resultant dyspnea. He found that some histaminase inhibitors did potentiate the histamine effect. Mongar and Schild (154) found that semicarbazide would potentiate the effect of histamine on guinea pig gut in concentrations greater than 10^{-9} g/liter and that the best concentration was 10^{-6} g/liter. Nielsen and Feigen (173) noted that semicarbazide could inhibit partially the destruction, by some soluble enzyme system, of the histamine released from the shocked guinea pig gut. Verschure (226) found no difference between the histaminase activity of sensitized and normal guinea pigs, but he found that the liver histaminase activity of summer guinea pigs was about half that of winter guinea pigs. Code *et al.* (47) challenged actively sensitized rats and found that the blood histamine rose to a maximum value of about 2 μg/ml within four minutes and decreased to about 1.6 μg/ml in ten minutes. They suggested that both histamine and histaminase were released simultaneously. However, Logan (135) obtained results in rats that indicated the histaminase activity did not appear until 6 to 13 minutes after challenge, and he found that the amount of histaminase activity could be reduced by ligation of the vessels of the gut. Graham *et al.* (75) found that the local tissue histamine content depended on the local histamine decarboxylase activity but that there was little or no relation between the histamine forming enzyme and the local histaminase activity. The metabolic pathways of histamine have been reviewed recently by Schayer (204).

1. Other potentiators of the anaphylactic reaction. Other materials have been found to be potentiators of the anaphylactic reaction, histamine release, or mast cell destruction. Moussatché (168) found that succinate and alpha-ketoglutarate potentiated the release of histamine from lung slices of actively sensitized guinea pigs. However, Diamant (57) found that succinate did not enhance the release from rat mast cells, and Chakravarty (41) found that succinate concentrations greater than 90 mM reduced the amount of histamine released. Spector et al. (216) found that sulfhydryl donors would restore the inhibition by sulfhydryl group blocking agents. The observation by many investigators that an injection of B. pertussis vaccine together with antigen into rats and mice increased their susceptibility to anaphylactic shock has caused some speculation about the reasons for this phenomenon. Mota has suggested that the presence of the pertussis organisms induces a "mast cell lytic" antibody (160-162). Sanyal and West (203) believe that the effect is to increase the sensitivity of the rats to histamine and serotonin. Cody et al. (48) found that when rats were sensitized with antigen and pertussis vaccine, and were challenged with antigen plus cortisone, the anaphylactic reaction was diminished. However, if cortisone was given at the same time as the sensitization injection, the rats became more susceptible to histamine. Niwa (175) has been able to separate and partially purify a material from the pertussis organism which increases the susceptibility of mice to histamine (175).

There are two enzyme systems which seem to be implicated in histamine release and mast cell degranulation. The results of Austen et al., mentioned above, have suggested that chymotrypsin is involved, and the

investigations of Uvnäs have suggested that another enzyme called lethicinase-A or phosphatidase-A is involved (222, 223). Keller et al. (112, 114) have found evidence that the mast cell esterase is not identical to bovine alpha-chymotrypsin, and by making antisera to both phosphatidase-A and alpha-chymotrypsin, they were able to show that the disruption of rat mast cells was not inhibited by the presence of the antisera. It may be that enzymes with properties common to chymotrypsin and to lethicinase-A are involved, or it may be that the difference in the test systems used may be the source of some of the discrepancy.

Summary.

The anaphylactic reaction is an immunophysiological response of animals or of isolated tissue to the union of antigen and antibody. The physiologic response is due to the liberation of pharmacologically active materials from cells involved with the antigen-antibody reaction. Animals can be sensitized by injecting them with antigen or by passive transfer of antibody from sensitized animals to normal animals; isolated tissue can be sensitized by exposure to dilute solutions of antibody.

The degree of passive sensitization depends on the kind and amount of antibody used. The source of the antibody is important; horse antibodies will not sensitize guinea pigs. Different fractions of antibody, separable by chromatographic or ultracentrifugal techniques, differ in their ability to sensitize tissues. The velocity of sensitization is proportional to the antibody concentration and to the temperature at which sensitization takes place. The uptake of antibody by tissues during sensitization depends on the bulk phase antibody concentration,

but is less temperature sensitive than the sensitization phenomenon. These observations suggest that sensitization is a separate process which follows antibody uptake. The presence of non-specific γ-globulins reduces the degree of sensitization.

The intensity of the anaphylactic reaction depends on the antigen concentration used, the physico-chemical environment of the challenged tissues and the degree of sensitization. For a given degree of sensitization, the intensity of anaphylaxis increases with increasing antigen concentration; at very high concentrations the anaphylactic reaction may be inhibited. If the tissues are challenged at a low temperature, then the intensity of the anaphylactic reaction is reduced. Oxygen or glucose are needed for the production of anaphylaxis. Metabolic inhibitors, such as sulfhydryl-blocking agents, reduce the severity of anaphylaxis. Calcium is required. The presence of substrates of chymotrypsin inhibits the reaction. Because of the wide variety of conditions which can affect the intensity of anaphylactic shock, there seem to be a great many steps between the union of antigen with antibody and the appearance of the anaphylactic reaction.

Although none of the details of the steps in the process of sensitization or the anaphylactic reaction have been described completely, the general features of the process have been determined. Sensitization seems to be a chemical reaction between antibody and the surface of cells. The union of antigen and fixed antibody starts a chain of enzymatic reactions, probably involving a chymotrypsin-like enzyme and a lethicinase, which culminate in the release of pharmacologically active materials. The study of the anaphylactic reaction in isolated cell systems probably will lead to more complete description of the individual steps in the reaction.

EXPERIMENTAL

The aim of the present studies was to estimate the minimal number of antibody molecules required to sensitize reactive cells in heart tissues. The estimate was made by combining the results of experiments relating antibody uptake and sensitization with certain assumed properties of the reactive cells in heart tissues. It was possible to formulate a relation between the amount of precipitating antibody present, or degree of purity, and the total γ-globulin in the sensitizing solution required to produce half-maximal sensitization of the tissues. Certain reasonable assumptions were made about the nature and number of the histamine releasing sites. The experimentally determined relation was used to estimate the number of antibody molecules required to sensitize a histamine releasing site.

In order to achieve the eventual aim, it was necessary to have a reliable means of estimating the intensity of the reaction. The release of histamine from shocked tissues was selected as the indicator of the reaction. It was necessary to be certain that not only could the compound itself be estimated accurately, but that the variations resulting from differences in the rates of production and destruction could be accounted for and corrected.

The experimental work may be divided into the following general sections:

1. Identification and estimation of histamine released from guinea pig heart tissues.

2. Measurement of the factors influencing the uptake of antibody by <u>in vitro</u> sensitized tissues (concentrations in the bulk phase, temperature).

3. Measurement of the factors influencing the degree of sensitization and the intensity of the subsequent anaphylactic reaction (antibody concentration, incubation time, antigen concentration, temperature, histaminase activity).

4. Sensitization of tissues with a mixture of two antibodies and estimation of the influence on the degree of sensitization to one antibody in the presence of the second.

The results of the experiments were used to estimate the minimal number of molecules required to sensitize reactive cells in heart tissue.

In order to avoid repetition of certain experimental procedures, a separate section was made which includes the procedures common to many experiments. The experiments are described and discussed as separate units to make their presentation smoother.

MATERIALS AND METHODS

Antigens.

Two preparations of ovalbumin were used during the course of the experiments to be described. The antigen used for the studies of the effect of sensitization procedure and challenge procedure on histamine release was ovalbumin crystallized by the method of Kekwick and Cannan (110). The ovalbumin used in the study of multiple sensitization was crystallized by the method of Sørenson and Hoyrup (215). Both preparations were dried from the frozen state, and were redissolved in physiological saline prior to use. Bovine serum albumin was obtained from Armour Laboratories.

Antibodies.

Rabbit antibody was prepared against each batch of antigen. The rabbits were given intravenous injections of the antigen twice weekly for six weeks. On the ninth day after the last injection the rabbits were bled by cardiac puncture and the blood was allowed to clot for several hours at room temperature and then overnight in the refrigerator. The antiserum was separated from the clot, and the γ-globulin fraction was precipitated by three-fold treatment with one-third saturated ammonium sulfate. The precipitated γ-globulin was redissolved in one per cent saline, and dialyzed against saline until the dialysate was sulfate free. The antibody preparations were stored in 10 ml serum bottles at 20 C until used.

Antibody to each of the three antigens was prepared. The precipitating titre of the antibody preparations was measured by the micro-precipitin

method of Lanni (126). Thirty-two per cent of the γ-globulin of the first antiovalbumin was precipitated by its specific antigen. Twenty per cent of the second preparation was precipitated by its specific antigen. The anti-bovine serum albumin (anti-BSA) contained 16.5 per cent precipitating antibody.

Other reagents.

All materials used were the purest available. Water was prepared by redistillation of water over alkaline permanganate in an all-Pyrex, continuous-flow system. Chenoweth's medium (45), the composition of which is listed in Table I, was used for perfusion and incubation of the heart tissues. The gas phase used was a mixture of 95 per cent oxygen, 5 per cent carbon dioxide. The final pH was between 7.3 and 7.4.

Cotton-acid succinate (CAS) and a mixture of 1 g $Na_3PO_4 \cdot H_2O$ to 6.25 g anhydrous Na_2SO_4 were prepared by the method of McIntire et al. (144); O-phthaldialdehyde (OPT) (California Corporation for Biochemical Research, Los Angeles, California) was recrystallized from ligroin as described by Shore et al. (211). Histamine diphosphate, obtained from Van Camp Laboratories, Terminal Island, California, was used as the standard for histamine determinations.

Histamine analysis.

Two techniques of histamine analysis were used. The first was a bioassay procedure using guinea pig ileal strips described by Feigen et al.(66). The bioassays were performed on the automatic bioassay

TABLE I

COMPOSITION OF CHENOWETH'S BALANCED SALT SOLUTION

NaCl	7.000 g
KCl	0.420
$MgCl_2 \cdot 6H_2O$*	0.428
$CaCl_2 \cdot 2H_2O$	0.318
Dextrose	1.800
$NaHCO_3$	2.100
H_2O	To make 1 liter

* In the form of a stock solution containing 17.1 g/100 ml of which 2.5 ml was used per liter of Chenoweth's.

device described by Boura et al. (32). The second was a chemical procedure based on a combination of the separation method of McIntire et al. (144) and the fluorometric procedure of Shore et al. (211). When compared, the bioassay and chemical methods agreed within about 10 per cent; the bioassay had the advantage that approximate values could be obtained quickly; the chemical method was insensitive to non-histamine smooth-muscle stimulants.

The chemical estimation procedure was as follows:

1. 5 ml of n-butanol were pipetted into a 15 ml ground-glass stoppered centrifuge cone.

2. 2 ml of sample were added.

3. Approximately 0.52 g of the phosphate-sulfate salt mixture was added using a precalibrated glass scoop; the cones were immediately stoppered and shaken by hand for 10 seconds to prevent the formation of a lump of salt at the tip of the cone.

4. The cones were shaken for 15 minutes in a horizontal action Kahn-type shaker.

5. After the shaking period, the cones were centrifuged at 2200 RPM in an International centrifuge.

6. 5 ml of the butanol phase were removed and passed over a 100 mg pad of CAS.

7. The CAS pad was washed with 3 ml of 95 per cent ethanol followed by 3 ml of distilled water.

8. Elution of the histamine from the CAS was accomplished by two successive elutions with 1 ml aliquots of 0.1 N HCl.

9. Regeneration of the columns was accomplished by washing twice with 7 ml of 0.1 N HCl followed by three washings with distilled water, one wash with 95 per cent ethanol and finally drying by passing air through the columns.

10. To the eluted histamine was added 0.2 ml of 2 N NaOH.

11. 0.1 ml of a 0.1 per cent (W/W) solution of OPT in absolute methanol was added. The reaction was permitted to proceed for 4.0 minutes.

12. The reaction was stopped by addition of 0.1 ml of 3 N HCl.

13. The fluorescence intensity of the OPT-histamine fluorophore was measured on an Aminco-Bowman spectrophotofluorometer (indicated excitation wavelength, 350 mμ, and emission wavelength, 450 mμ).

For further details of the chemical method, especially the effect of varying certain steps in the procedure, see reference (229).

Tissue Preparation.

Male guinea pigs, weighing between 300 and 700 g were the source of the tissues used in the experiments. Two types of experiments were performed; the first involved the use of perfused whole hearts, and the second used isolated fragments of heart tissue, mainly atrial tissue.

The hearts were prepared for the perfusion studies as follows:

1. The animal was primed with 2 mg/kg of sodium heparin injected intraperitoneally about 15 minutes before the start of the dissection.

2. The animal was killed by a blow to the base of the skull; the thoracic cage was opened; the pericardium was removed; a stout linen thread was passed around the aorta near its origin.

3. A small flap was cut in the aorta and a glass cannula connected to a reservoir of warm oxygenated Chenoweth's solution was inserted; the thread was tied around the cannula.

4. The inferior vena cava was severed and the heart was perfused via the coronaries until it was free of most of its blood.

5. The heart was removed from the carcass, and mounted on the Anderson Heart Perfusion Apparatus (Metro Industries, Long Island, New York); perivascular tissue was trimmed from the heart.

6. A nylon thread was tied to the apex of the heart and connected to an ink-writing kymograph when a record of mechanical events was desired.

7. Coronary flow was measured by collecting the perfusate which had passed through the heart for a specific time. This flow included both the true coronary flow and some leakage flow.

8. At the end of the experiment, the heart was removed from the Anderson apparatus, and its chambers were opened; it was blotted with filter paper, placed in a covered weighing bottle, and weighed. Dry weights were obtained by drying the tissues in an oven at 105 C for 24 hours.

Two procedures were used to isolate atrial tissues. In the first, the hearts were prepared as in steps 1 through 5 above. Then the atria were trimmed from the ventricles and placed in 200 ml flasks of oxygenated Chenoweth's at the desired temperature. The second method involved removing the hearts immediately after the thorax was opened, and placing them under a continuous flow of oxygenated Chenoweth's; the atria were trimmed from the ventricles. Several experiments involved the use of groups of tissue;

the prepared atria were tied with nylon thread in groups of three. A small loop of thread was left near the tissue group. The tip of a piece of capillary tubing was inserted into the loop. The gas line was connected to the other end of the capillary; the free end of the thread was taped to the capillary, also. In this way, the group of tissues could be handled conveniently, and transferred from tube to tube with little effort. The sensitization was carried out by placing the tissue groups in an appropriate volume of antibody solution at the experimental temperature for the desired time. After incubation, the tissues were washed by transfer through a series of volumes of antibody-free solution; the tissues were challenged by putting the group into an antigen solution. After the experiment, the tissues were blotted with filter paper and dried in a vacuum dessicator. Certain variations of the above procedures were used in some of the experiments. These variations are included together with the description of the experiment.

EXPERIMENTS

Relative Weight Composition and Histamine Content of Guinea Pig Heart Tissues

Measurements were made of the histamine content of heart tissues and the fraction of the histamine content released by anaphylactic challenge. The weight fraction of the heart comprised by its major components was determined.

Methods.

Hearts were dissected into three components: the aortic stump, the atria, and the ventricles. They were blotted gently on filter paper and placed in covered weighing bottles. After their wet weights were measured, the bottles were opened and placed in an oven at 105 C and dried to a constant weight. The ratio of wet-to-dry weights was calculated and is given in Table II. We calculated the weight fraction of each heart occupied by each of its three components, and computed the relative make-up of a "standard" guinea pig heart. Table II includes the results of the computation.

We perfused the hearts of normal guinea pigs until they were free of blood. The aorta, atria, and ventricles were separated, were placed in containers of distilled water at room temperature, and were minced to insure access of the water to all portions of the tissues. The tissues were allowed to soak for 30 minutes. At the end of the soaking time, the fluid was filtered, a portion was saved for histamine analysis by the fluorometric method described above, and the tissue fragments

TABLE II

WEIGHTS AND RELATIVE COMPOSITION OF THE "STANDARD" GUINEA PIG HEART

	Wet weight/dry weight
Atria	5.47 ± 0.31 (n = 25)
Aorta	3.36 ± 0.53 (n = 22)
Whole heart	5.9 ± 2.00 (n = 18)

	% of wet weight of "standard" heart
Atria	11.0
Aorta	3.5
Ventricles	85.5

were dried to constant weight. The histamine released, in moles per g of dry tissue, is listed in Table III.

We actively immunized 12 guinea pigs by intraperitoneal injection of two 20 mg doses of ovalbumin in 1 per cent saline. The injections were three days apart. Seven weeks after the second injection, the animals were sacrificed, and their hearts were perfused until free of blood. The aorta, atria, and ventricles were divided, and minced into separate containers of oxygenated Chenoweth's solution, maintained at 37 C. Ovalbumin dissolved in Chenoweth's was added to make the final concentration 1 mg per ml. The tissues were incubated 15 minutes. After incubation, the fluid was filtered, an aliquot portion was saved for fluorometric histamine analysis, and the tissue fragments were dried to constant weight. The mean and range of values of the histamine released are shown in Table III. Control experiments, substituting normal animals for the immunized animals, showed a spontaneous release of 3-5 per cent of the release from immunized animal tissue. The figures of Table III are uncorrected for spontaneous release.

Results and Discussion.

Distilled water causes mast cells to disrupt and release their histamine (195). Most of the histamine in tissues can be released by distilled water treatment (143).

With the aid of the wet-dry ratio and composition of a "standard" 1 g heart, we can relate our findings to the reports of other experimenters. These reports for the whole heart and aorta (29, 36, 42, 147, 206) show values of 3-4 μg of histamine base per g of whole heart, and

TABLE III

HISTAMINE RELEASE FROM HEART TISSUES BY DISTILLED WATER AND ANTIGEN

Tissue	Histamine release (moles/g dry wt x 10^7) Distilled Water	n	Actively sensitized tissues	n
Atria	4.45(2.33-7.95)	11	4.5(2.85-5.37)	12
Aorta	6.12(2.97-9.02)	8	1.20(.3 -3.2)	12
Ventricles	1.57(.79-2.06)	7	.49(.31- .79)	12

( ) = range

10-30 μg per g of aorta. We obtained a value of 6.1×10^{-7} moles/g dry weight of aorta, which, when multiplied by the molecular weight of histamine (1.1×10^{2}) and divided by the wet to dry weight ratio for aorta of 3.36, yields 20 μg of base per g of wet aorta. In our preparation, a 1 g "heart" contained 110 mg of atrial tissue, 3.5 mg of aortic tissue, and 855 mg of ventricular tissue. The dry tissue weights, obtained by dividing by the wet to dry ratio, are 20 mg, 1.0 mg, and 183 mg. The contributions of each to the distilled water histamine release from a whole heart, found by multiplying the dry weight by the release value, are .98 μg, 0.06 μg, and 3.1 μg, for a total of 4.1 μg per 1 g heart. In the case of anaphylactic histamine release, the figures are 0.98 μg, .01 μg, and 1.1 μg for atrial tissue, aortic tissue, and ventricular tissue, respectively. This combines to about 2.1 μg/g of whole heart, or about 50 per cent of the releasable histamine. This value is comparable to the values mentioned in the literature.

Challenged atria release essentially all of their histamine content, while the other two tissues release only 20 to 30 per cent. The latter value is that which has been reported for whole hearts. The high sensitivity of the atria compared with the other tissues, means that the atria contribute almost half of the measured release from a challenged heart and comprise about one tenth of the weight of the heart. The atria seem to be the only tissues, except perhaps for mast cell tumors, which release all of their histamine during an anaphylactic reaction. This property was utilized in the later experiments on double sensitization.

The Binding of Antibody to Heart Tissues as a Function of Concentration and Temperature

The uptake of radioactive antibody by heart tissues was studied in order to determine the relation between the bulk-phase concentration used to sensitize tissues and the amount retained by the tissues. The experiments were done at 37.5 C for whole hearts and at 32.7 and 27.2 C for isolated atrial tissue and aortic tissue.

Methods.

Heart tissues were exposed to the antibody by perfusion and by soaking. Antiovalbumin was iodinated with I^{131} by the method of McFarlane (142). Approximately 1.5 mole of iodine was coupled per mole of γ-globulin. We used a Nuclear-Chicago single-channel analyzer and a well-type scintillation detector to measure the radioactivity of our samples.

In the first of the series of experiments, the hearts were perfused with radioactive antibody on the Anderson apparatus. At the end of the adsorption phase the hearts were washed with antibody-free Chenoweth's solution until an amount equal to twice the volume of antibody solution had passed through the hearts. At the end of the washing phase the hearts were placed in a beaker of cold (about 4 C) Chenoweth's solution and the radioactivity contained on the heart was measured with the scintillation counter. After the count was made, the hearts were restored to the Anderson apparatus and were allowed to re-establish their normal activity before being challenged. When the rate had returned to its precount values the hearts were challenged with 1 mg

of ovalbumin instilled in the aortic cannula in a volume of 0.5 ml. Perfusates were collected before the challenge as well as at various intervals during the height of the physiological reaction to the specific antigen. When the heart had recovered from the effects of the anaphylactic reaction, it was re-challenged with the same dose of antigen as had been used initially. None of the preparations used reacted to a second dose of antigen. In order to demonstrate that the failure of the second response had resulted from specific desensitization rather than from tachyphylaxis to histamine, a preparation was tested with 0.1 ml of 10^{-3} M histamine made up in Chenoweth's solution. The counts of the perfusate taken before the challenge showed that the amount of radioactivity being washed off represented a small fraction of the amount retained on the tissues and that the rate of change of washoff was small. The amount of radioactivity present in the perfusate during challenge increased slightly in the first 2 minutes of the reaction and then returned to the pre-challenge value. This transient increase also was noted when the heart was treated with epinephrine, and it was presumed that the transient increase was not due specifically to the antigen-antibody reaction which caused the anaphylactic reaction of the heart. After the challenge the hearts were removed from the Anderson apparatus; the atria were separated from the ventricles; the radioactivity of both were measured; and the weights of the blotted tissues were recorded.

The binding of radioactive antibody to the separated atria and ventricles was measured in a second experiment. The hearts were perfused with labelled antibody, as in the previous experiment. At the end

of the wash phase, the atria were trimmed from the ventricles, and the radioactivity of both atria and ventricles was counted. After the atria were removed the ventricles were sliced approximately 0.4 mm thick. The tissues were placed in separate containers of oxygenated Chenoweth's after the counting procedure and were equilibrated at 37 C for 10 minutes. Ovalbumin was added to each container to make the concentration 1 mg/ml. After incubating the tissues in the challenge solution for 15 minutes, they were removed, the residual radioactivity was counted, and their wet and dry weights were measured. The challenge solutions were saved for histamine analysis.

The effect of temperature and concentration on the physical binding of antibody to atria and aorta was studied. The hearts of normal guinea pigs were removed; the atria were trimmed quickly from the ventricles and placed in an oxygenated flask of Chenoweth's at 37 C. Both the stump of the aorta, used for the perfusion, and a portion of the thoracic aorta were removed and placed in a flask of oxygenated Chenoweth's at 37 C.

Radio-iodinated antibody was made to concentrations of 14.4, 30, 250, and 500 μg/ml. Centrifuge cones containing 25 ml of the antibody solution were used as incubation containers. We incubated groups of three atria and aorta in separate containers of the four antibody solutions at both 27.7 C and 32.4 C for 1 hour. Upon completion of incubation, the tissues were rinsed in three changes of Chenoweth's, and their radioactivity was measured. After the ccunting procedure, they were transferred to the 37 C bath, and challenged with a 1 mg/ml antigen

solution. The wet and dry weights, and residual radioactivity of the tissues were determined. The challenge solutions were saved for histamine analysis.

Results and Discussion.

There seemed to be no significant difference in the uptake of antibody by whole hearts, the separated ventricular tissues, or the atrial tissues. Therefore the results of the binding study of these tissues at 37.5 C were grouped together and are shown in Figure 1.

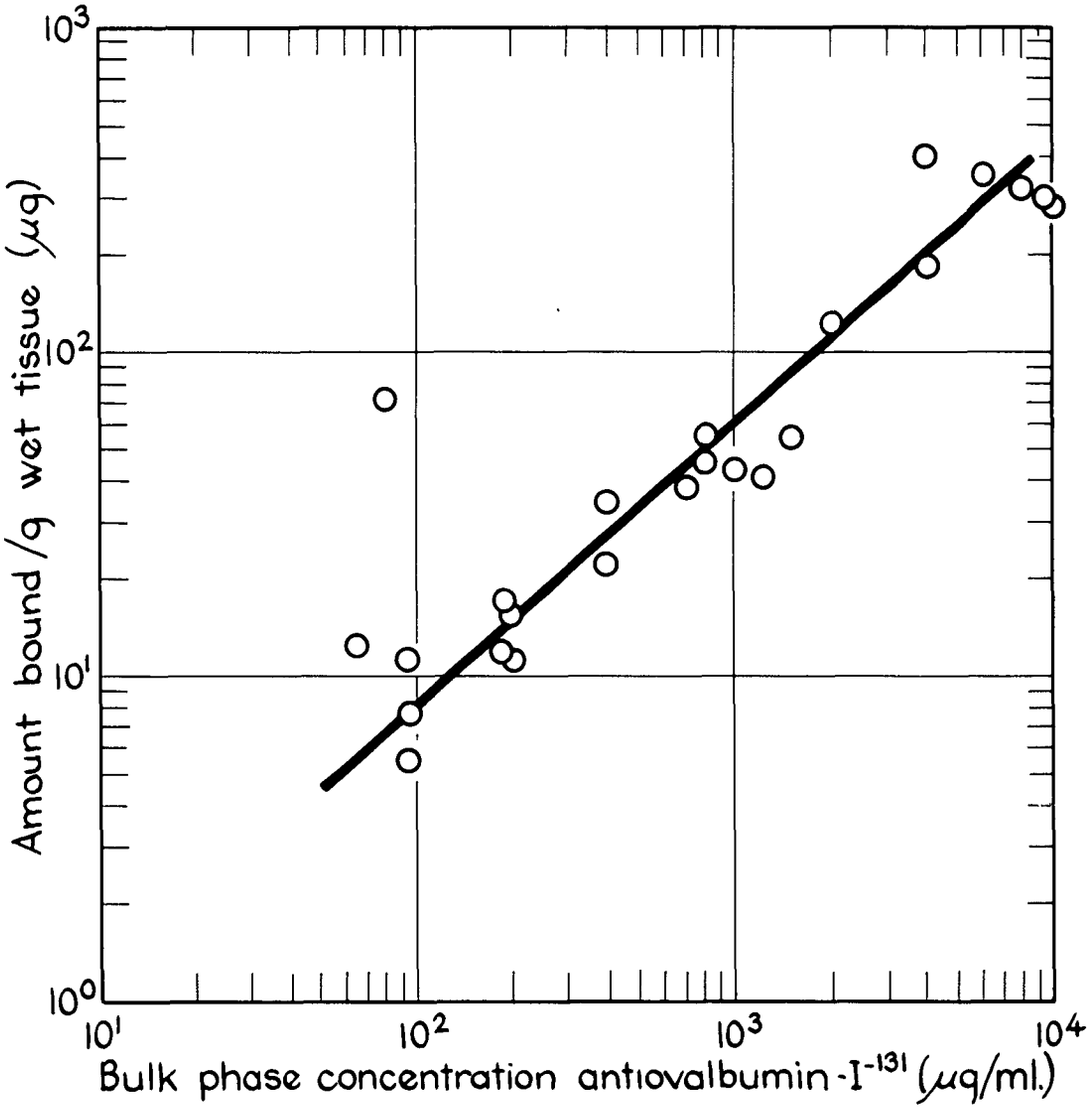
The amount of antibody retained by heart tissues is proportional to the bulk-phase concentration of antibody used to sensitize the tissues. The slope of the binding curve is nearly unity and has a value of 1 μg of antibody per g of wet tissue per 10 μg antibody per ml of solution. This value is comparable to reports by other investigators for guinea pig ileum (100, 102, 174), chopped lung (37), and rat mesentery (89). The radioactive tracer technique we used did not permit us to distinguish between antibody actually bound or adsorbed to tissues and that in the extracellular spaces. However, perfusion, soaking, and challenge did not remove a substantial fraction of the radioactivity retained by the exposed tissues after incubation except for material which appeared in the early part of the washing phases. Both the resistance to removal of antibody by washing, and Brocklehurst's (37) report that the addition of non-radioactive γ-globulin to the wash fluid markedly increased the rate of elution of radioactive material are strong evidence that much of the residual antibody is retained firmly by the tissues but is exchangeable with other γ-globulins, and is not free in the extracellular space.

FIGURE 1

BINDING OF RADIO-IODINATED ANTIBODY

TO IN VITRO SENSITIZED HEART TISSUE AT 37 C

BINDING OF RADIO-IODINATED ANTIBODY TO HEART TISSUE AT 37 C



However, there is some evidence that there is a fraction of the antibody retained by the tissues that is not fixed as firmly. Table IV shows the appearance of radioactivity in the perfusates taken from the heart perfused with 702 μg antibody per ml. Both histamine and epinephrine produced a transient efflux of activity; specific antigen also increased the rate of wash-off. The increased mechanical activity of the heart under drug stimulation may bring about a faster exchange of fluid between the blood vessels and extracellular space, resulting in a transient appearance of radioactivity. The effect of antigen may be due to the mechanical stimulation during the anaphylactic reaction as well as to the specific union of antigen and antibody. This specific effect might be to weaken the bond between antibody and cell in a few cases, causing the appearance of radioactivity in the perfusate. The amount of material retained is many times the amount removed during the latter part of the washing phase, or by antigen, or drug action.

The mechanism of uptake of antibody must involve a surface adsorption step; attachment of antibody to a cell reflects a physical process. Whether the subsequent transfer of antibody into the cell occurs and is important in the mechanism of anaphylaxis is not known yet, but adsorption still would be the primary event. The mechanism of physical adsorption was described by Langmuir (125). The Langmuir adsorption isotherm relates the bulk phase concentration, c, to the amount, a, of material bound to the substrate.

$$c/a = k_1 + k_2 c \quad . \qquad [1]$$

TABLE IV

EFFECTS OF SPECIFIC AND NONSPECIFIC AGENTS ON THE EFFLUX OF LABELLED ANTIOVALBUMIN*

Time After Treatment (min)	Counts/min-ml perfusate			
	Histamine (0.08 mg)	Ovalbumin (challenge) (1 mg)	Ovalbumin (re-challenge) (1 mg)	Epinephrine (0.05 mg)
0	4,470	650	69	56
1	9,600	600	66	204
2	12,000	950	93	151
3	4,750	590	62	96
5	3,400	525		109

* Protocol from heart perfused with 702 μg protein/ml.

This simple expression has been shown to be valid in a great many experiments performed at low concentration. Deviations from linearity will occur at high concentrations because multiple layers of material will form and will change overall surface properties.

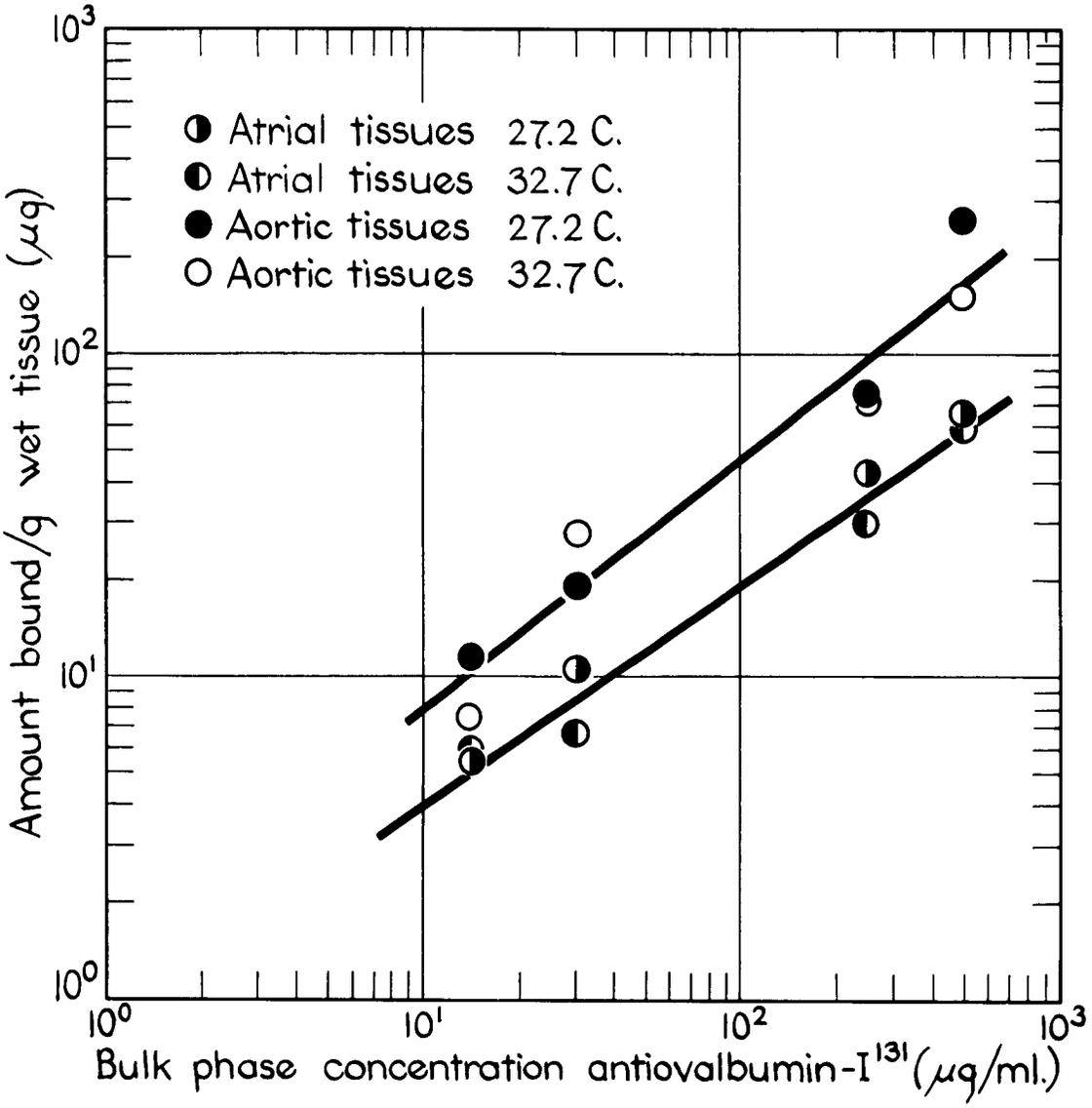
Adsorption studies on complex systems like tissue can give misleading results. The extracellular space can hold antibody in solution which may not be removed readily by washing. Variability in the retention of the extracellular fluid might cause the apparent adsorption curve to be distorted. Application of the Langmuir relation [1] to the data of Figure 1 gave rather scattered results, which may have been due to the above reasons. However, the adsorption relation has been used successfully by Nielsen et al. (174); they used in vitro sensitized ileal strips. That system may be more applicable to adsorption analysis.

The studies of atrial tissues and aortic strips shown in Figure 2 show that physical binding to these tissues also has a low temperature coefficient. The lines for aortic tissue are displaced somewhat above those for atrial tissues; the displacement may be due to a different weight-to-surface ratio. However, it is not possible to distinguish a temperature effect on the binding. The data points for the two temperatures lie quite close to each other, and there is no apparent order among them. This statement applies to the data for both atrial and aortic tissues. Brocklehurst et al. (37) reported that changing the incubation temperature from 37 C to 0 C reduced the uptake of tagged antibody by chopped guinea pig lung. The uptake was reduced to half its value at 37 C. Feigen et al. (64) reported that uptake of radioactive antibody by guinea pig ileal strips was essentially the same whether incubation took place at 37 C, 27 C, or 20 C.

FIGURE 2

TEMPERATURE DEPENDENCE OF BINDING OF
ANTIBODY TO HEART TISSUE

TEMPERATURE DEPENDENCE OF BINDING OF ANTIBODY TO HEART TISSUE



Factors affecting histamine release from isolated heart tissues.

We studied the effects of the following on the release of histamine from isolated heart tissues: antibody concentration, antigen concentration, incubation time, and challenge temperature.

Methods.

Antibody concentration: Sensitization of heart tissues was performed using the Anderson perfusion apparatus and by soaking isolated, normal atria in antibody solution.

In vitro sensitization of whole heart was performed using antibody concentrations of 100 μg/ml and 50 μg/ml. The hearts were perfused with a total of 150 ml of antibody solution and then perfused with normal Chenoweth's solution until the rate, coronary flow, and amplitude of contraction had returned to the presensitization values. The hearts then were challenged with 0.5 ml of a 2 mg/ml antigen solution instilled into the aortic cannula. After the hearts had recovered from the reaction, a second challenge was made to verify the desensitization and eliminate the possibility that the antigen had non-specific effects upon the heart. Following the second challenge, a dose of histamine was given to test whether the loss of reactivity was due to tachyphylaxis to histamine. The hearts failed to react to the second challenge and always demonstrated a typical response to the histamine injection.

In vitro sensitized atrial tissues were used to study a wider range of antibody concentration. The isolated atria were prepared according to the second method described in the Materials and Methods section.

The incubation time was 2 hours; the incubation volume was 30 ml. Three 5-minute washes in 30 ml of antibody-free Chenoweth's solution were performed after incubation. The tissues were challenged for 10 minutes in 25 ml of Chenoweth's with ovalbumin added to a final concentration of 0.92 mg/ml. The tissues were dried, and the challenge solution saved for chemical histamine assay.

Incubation time: The procedure used for the study was similar to that described in the previous section. Two antibody concentrations were used for the incubation time studies: 150 and 10 μg/ml; challenge concentrations were 450 and 800-1000 μg/ml respectively.

Antigen concentrations: A group of atria were sensitized for one hour in 100 μg/ml antibody solution in the usual manner. They were challenged initially with low antigen concentrations. After the first challenge, they were washed in regular Chenoweth's 3 times, for 5 minutes each time. The tissues then were placed in Chenoweth's solution with antigen added to a concentration of 44 μg/ml; the reaction was allowed to proceed for 10 minutes. The tissues were removed and dried to constant weight, and the challenge solutions were assayed for histamine. A second group of tissues was sensitized and challenged with antigen concentration ranging from 0.044 μg/ml to 903 μg/ml. These tissues were not challenged twice.

Results and Discussion.

Antibody concentration and incubation time: The results of the studies of the effect of antibody concentration on histamine release from perfused whole hearts are listed in Table V. Included in the table

TABLE V

HISTAMINE RELEASE FROM SENSITIZED HEARTS FOLLOWING ADMINISTRATION OF ANTIGEN

Antibody in bulk phase (μg/ml)	Release of Active Substance expressed as Histamine (moles/g/min)	Number of Hearts Tested
63 - 9,400	$1.19 \times 10^{-8} \pm 0.18 \times 10^{-8}$*	7
100	$0.96 \times 10^{-8} \pm 0.14 \times 10^{-8}$*	20
50	$0.49 \times 10^{-8} \pm 0.09 \times 10^{-8}$*	26
actively sensitized**	$1.10 \times 10^{-8} \pm 0.21 \times 10^{-8}$*	18

* Standard deviation

** Data from Feigen et al. (66)

are the data from some of the binding experiments, and, for comparison purposes, the values for actively sensitized tissues reported by Feigen et al. (66). The maximum rate of release of histamine from the challenged tissues was about 1 x 10^{-8} moles/g (wet) tissue/min. This rate was obtained from tissues perfused with antibody concentrations ranging from 63 μg/ml to 9.4 mg/ml, and from the actively sensitized group of tissues. The rate of histamine release was reduced to about one-half of the maximum when the antibody concentration was 50 μg/ml.

Anaphylactic shock produces changes in the mechanical events of the heart as well as causing histamine release. The effects of shock and of added histamine are parallel for both rate and amplitude. Although the release of active material is reduced if the bulk phase concentration is dropped from 100 to 50 μg/ml, it can be seen from Table VI that the increments in heart rate and in the amplitude of contraction appear to be effectively equal for both categories, as well as for histamine. The observation that challenge increased these variables by so precise a degree in all cases evidently results from the fact that the tachycardia is probably atrial (66, 186) in origin and that the increased ventricular frequency is limited, in the present experiments, by the inability of the atrioventricular bundle to conduct impulses at a rate greater than about 36 to 40 per cent above normal. This was the maximum increment before atrioventricular block became evident. Since a relationship exists between rate and amplitude (218), it is not surprising that, as the rate becomes limiting, the extent of ventricular contraction should attain a value determined by that rate.

TABLE VI

ACTION OF SPECIFIC ANTIGEN AND HISTAMINE ON SENSITIZED HEARTS

Antibody in Bulk Phase µg/ml	Tested with	Heart Rate			Amplitude			No. Hearts Tested
		Control	Challenge	% Change	Control	Challenge	% Change	
		beats/minute			millimeters			
63,- 9,400	O	225 ± 17*	305 ± 14*	+ 36	23 ± 3.0*	36 ± 6.0*	+ 57	7
100	O	206 ± 6	280 ± 5	+ 36	28 ± 1.6	44 ± 3.2	+ 57	20
	H	202 ± 5	274 ± 4	+ 36	23 ± 2.1	46 ± 3.0	+100	
50	O	201 ± 5	282 ± 6	+ 40	22 ± 1.7	37 ± 2.4	+ 67	26
	H	199 ± 4	272 ± 9	+ 37	19 ± 1.6	38 ± 2.4	+103	
actively sensitized**	O	207 ± 13	257 ± 16	+ 24	26 ± 2.1	56 ± 5.0	+113	18
	H	184 ± 14	216 ± 18	+ 17	16 ± 2.6	34 ± 4.4	+120	

* Standard deviation
** From Feigen et al. (66)
O = Ovalbumin
H = Histamine

The degree of sensitization of tissues depends on the antibody concentration used to sensitize the tissues and on the duration of the incubation period. The lower limit on the amount of antibody required to sensitize guinea pig tissues has not been determined directly, but the limit is probably in the μg per ml range. For example, Kabat and Boldt (106) were able to passively sensitize intact animals with as little as 5 μg/ml, and Binaghi et al. (22) sensitized ileal strips by soaking them in 2 ng/ml of antibody nitrogen for several hours.

It is reasonable to presume that the degree of sensitization is proportional to the amount of antibody in the tissue. Nielsen et al. (174) reported that the magnitude of the Schultz-Dale reaction paralleled the amount of radioactive antibody bound. Later work (63) has suggested that sensitization can be increased by extending the incubation time in the absence of free antibody in the incubation solution. Brocklehurst et al. (37) have reported that the uptake of antibody by lung tissue continues to take place long after sensitization is complete. There does seem to be good reason to suppose that uptake and sensitization are not synonymous.

It is the current consensus that sensitization can be separated into two parts. The first is an adsorption of antibody by the tissue; the second is a fixation process during which the antibody becomes attached to a sensitizable site. It seems reasonable that less than 1 per cent of cells in sensitized tissue are involved directly with the anaphylactic release of histamine (or other active materials). There seems to be no preferential uptake of antibody by sensitizable

cells (91), so that 99 per cent of the antibody measured in binding studies is not attached to reactive tissues. Therefore it may be difficult to relate bound antibody directly with the degree of sensitization. However, the sensitization proceeds at a different rate from uptake, so that it is possible to separate the processes.

The way in which sensitization occurs suggests that the sensitizable site and antibody molecules have a strong affinity for each other. The observation that sensitization increases in the absence of free antibody in the incubation solution may be accounted for by considering that the γ-globulin molecules can dissociate from non-reactive sites and migrate to reactive sites to which they are more firmly bound. The migration of antibody molecules from sensitized red cell to non-sensitized cell has been observed by Bowman et al. (33). The process of binding to a reactive site probably has a high activation energy, which could account for the slow but progressive increase in sensitization even in the presence of low antibody concentrations. Another possible implication of the kinetics of sensitization is that the reactive cell may have several sensitive sites, of which perhaps only one need be covered by antibody for sensitization to be produced. This would account for the observation that complete sensitization can occur before antibody uptake is completed. It also would account for the fact that complete desensitization did not occur when sensitized tissues were perfused with non-specific homologous γ-globulin. The latter process might exchange sensitizing antibody with non-specific γ-globulin at some of the sites but not all, and the tissues would remain sensitized. It also would account for the observation that tissues can be sensitized to several antigens at one time.

There are two intersecting curves in Figure 3; the curve for the 2 hour incubation experiment shows less relative sensitization at high antibody concentration than the 1 hour curve. This may be due to non-specific factors such as denaturation of the antibody by prolonged gassing of the incubation solution and coating of the tissues with denatured protein. However, at low concentrations, the effect of extending the incubation period is observable.

Figure 4 shows this more clearly. The slope of each line, which is proportional to the velocity of sensitization, increases with increasing concentration. If each slope is expressed as per cent of maximum sensitization per minute of incubation time and the maximum is chosen to be 5×10^{-7} M/g dry tissue, then the initial slope of the 10 μg/ml curve is about 1/2 per cent per minute, and the slope of the 150 μg/ml curve is about 2 per cent per minute. Different antigen concentrations were used, but in the next section it is shown that there is little difference between the histamine released by antigen concentrations between 450 μg/ml and 900 μg/ml. The ratio of the antibody concentrations is about 15:1, and the ratio of the velocities is about 4:1, suggesting that the velocity of sensitization is proportional to the square root of the antibody concentration. There are only two data points available for this suggestion so that no elaborate theory could be proposed, but it is interesting to note that Binaghi et al. (23) reported the same relation for the sensitization of guinea pig ileal strips.

HISTAMINE RELEASE AS A FUNCTION OF ANTIBODY CONCENTRATION

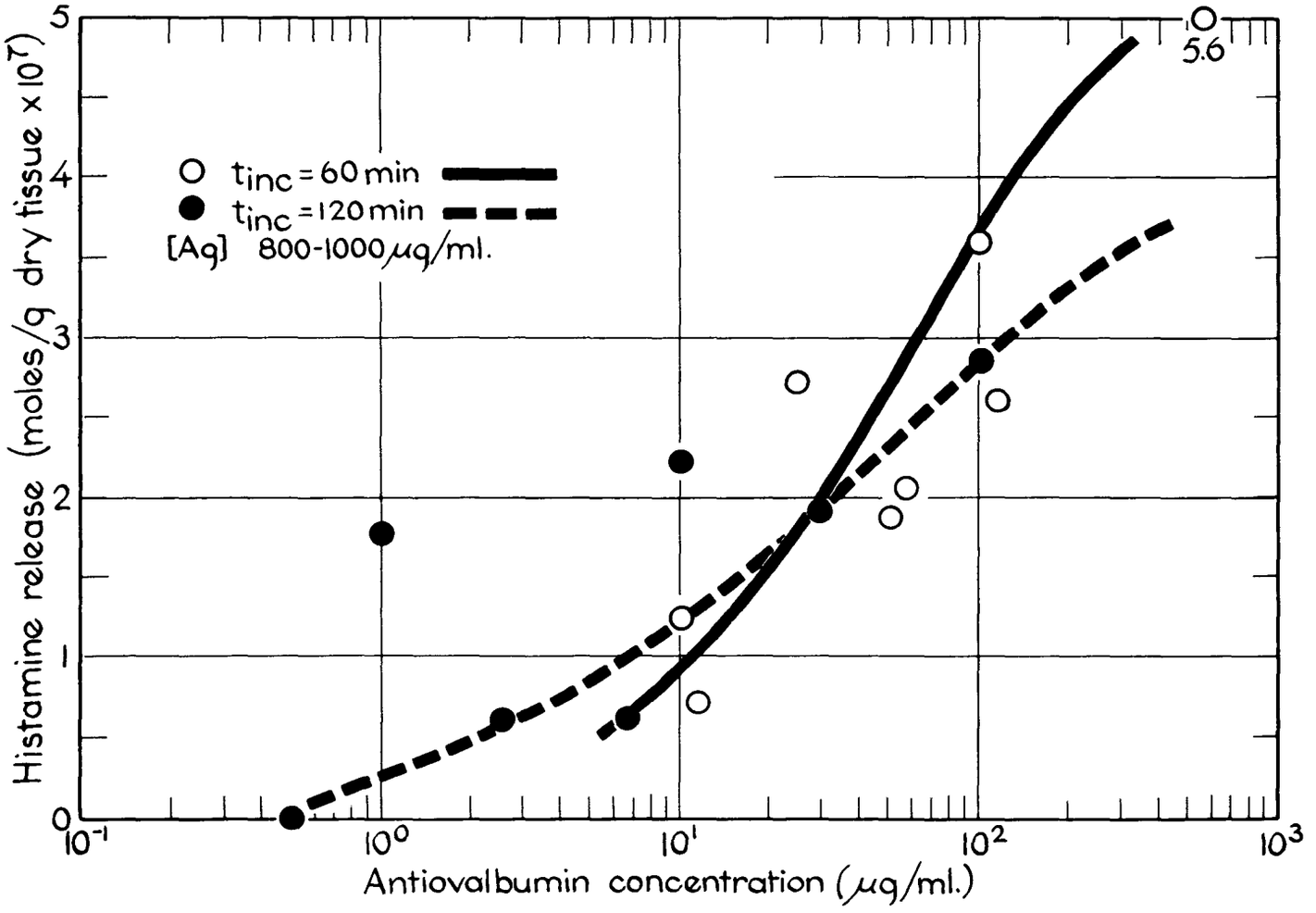
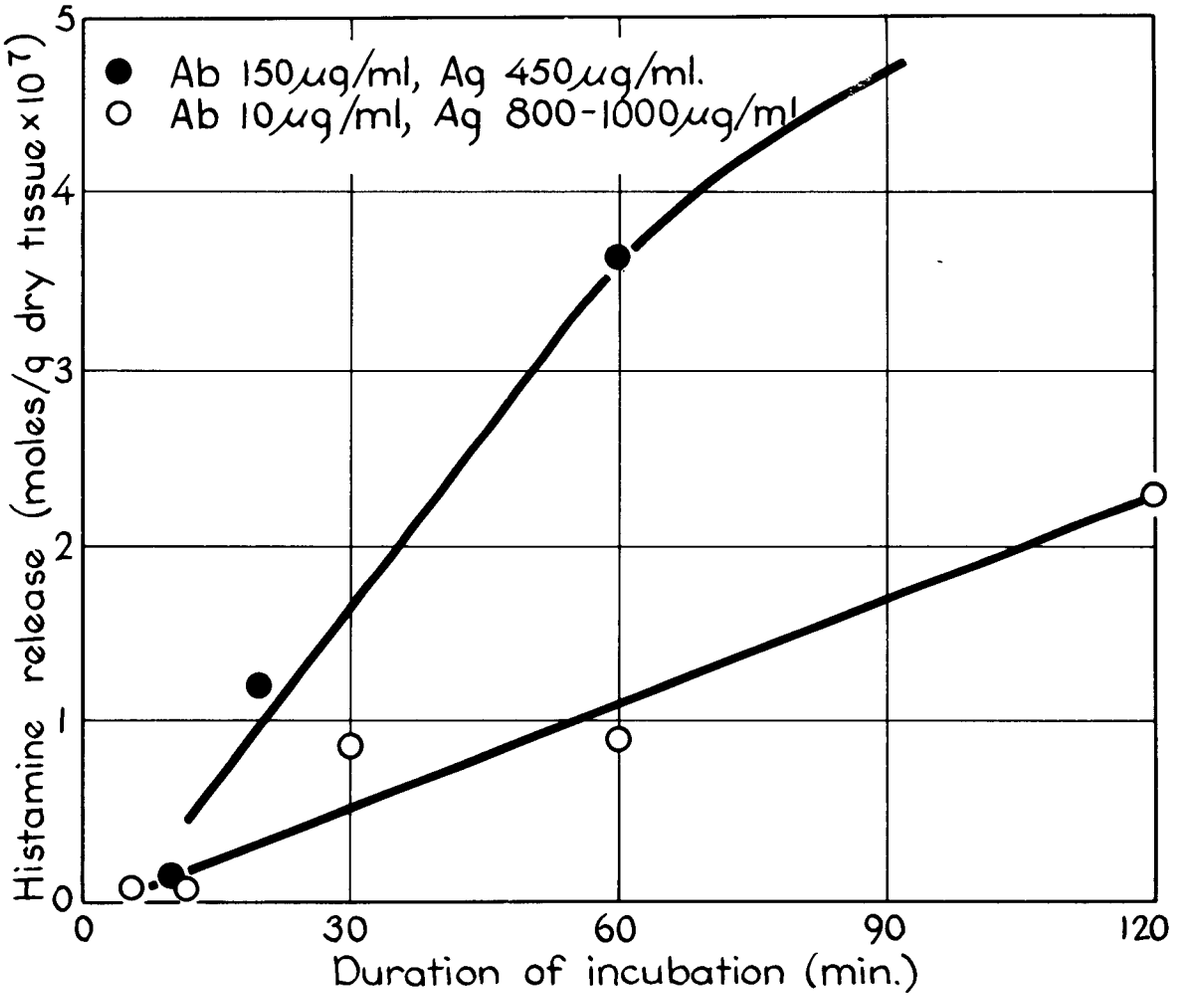


FIGURE 4

HISTAMINE RELEASE FROM IN VITRO SENSITIZED ATRIA
AS A FUNCTION OF INCUBATION TIME

HISTAMINE RELEASE AS A FUNCTION OF INCUBATION TIME



Antigen Concentration: The amount of histamine released from twice challenged atria is summarized in Table VII. The release resulting from the initial challenge increases with increasing antigen, and the release resulting from the second challenge decreases in antiparallel fashion. These results are similar to those reported for intact animals (189), the Schultz-Dale reaction (63), and the PCA (200). In a somewhat similar experiment, Liacopoulos (129) soaked sensitized ileal strips in 0.016 μg antigen per ml for various lengths of time and found that when the strips were challenged with a high concentration of antigen after the soaking period, the magnitude of the Schultz-Dale reaction declined as the soaking period was prolonged. It appears that if low antigen concentrations are used, submaximal reactions occur, and partial desensitization may occur. The latter suggestion may explain the observation that the total histamine released by both challenges was at a minimum when the initial challenge concentration was approximately 0.5 μg/ml. The minimum was observed for the Schultz-Dale, also (63).

Figure 5 shows the amount of histamine released from tissues challenged with various antigen concentrations. There seems to be no optimal concentration nor any drop-off at the high concentration end of the curve. Some investigators have used very large antigen concentrations and have found that inhibition of the reaction does occur (37, 131). The common experience is that the degree of the anaphylactic reaction is proportional to the challenge dose of antigen used, except when the dose is very high. The anaphylactic reaction is different from the in vitro precipitin test and probably would not be expected to show the same response to variations in the ratio of antigen to antibody as would the precipitin reaction.

TABLE VII

ATRIAL HISTAMINE OUTPUT IN RESPONSE TO SUBMAXIMAL DOSES OF ANTIGEN

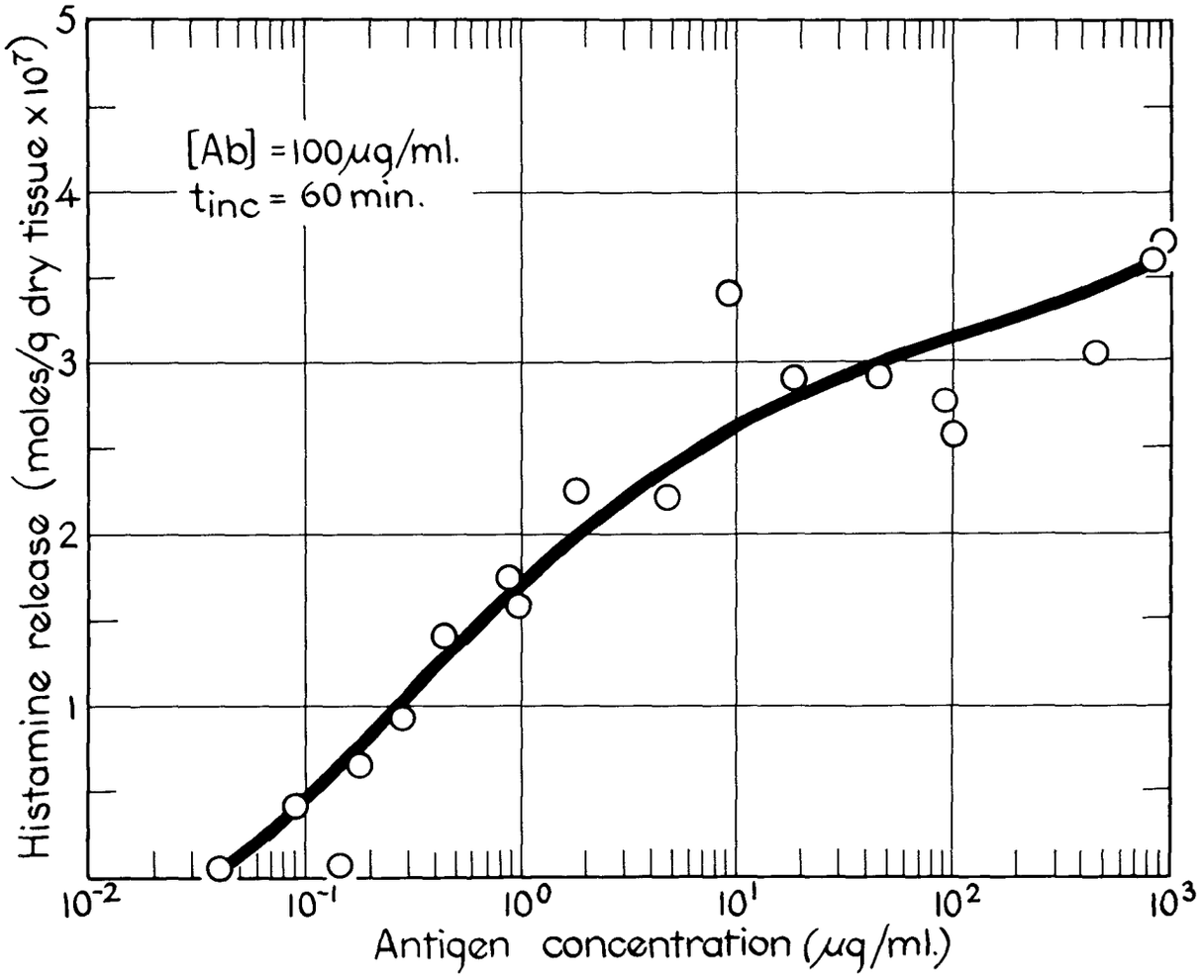
Antigen Concentration (μg/ml)	Release (moles/g x 10^7 dry weight)	
1st challenge	1st challenge	2nd challenge
0.086	0.039	0.70
0.180	0.51	3.27
0.44	1.31	1.20
0.86	1.38	0.26
2.98	0.62	0.00
14.0	5.11	0.00
36.0	3.53	0.00
73.0	3.66	0.00

Antibody concentration = 100 μg/ml
Antigen concentration (2nd challenge) = 44 μg/ml
Time of incubation = 1 hour
Time of challenge = 10 minutes
Temperature for incubation and challenge = 36.8 C

FIGURE 5

HISTAMINE RELEASE AS A FUNCTION OF ANTIGEN CONCENTRATION

HISTAMINE RELEASE AS A FUNCTION OF ANTIGEN CONCENTRATION



Although reports about the relation between the sensitizing dose and the dose required to produce systemic anaphylaxis conflict (49, 50, 106, 107), investigators using isolated systems have found that if a larger dose of antibody was used to sensitize the tissue, a concomitantly smaller dose of antigen was required to produce a given magnitude of reaction (42, 100, 127, 129, 171). Their results suggest that the reactive cells respond only when the rate of stimulation of sensitized sites by the combination of antigen and antibody exceeds a certain value. Let us assume that a reactive cell has several places which can be sensitized when antibody molecules are attached suitably, and let us assume that formation of an Ag_2Ab complex is part of the stimulus which starts the anaphylactic reaction. If it were required that several sensitized sites be hit by antigen within a certain time period for the reaction to occur, then several of the observed effects can be explained. If sensitization were carried out in the presence of a high antibody concentration, then many sensitizable sites would be filled, and the probability that antigen molecules would hit a sensitized site would be high; consequently, few molecules would be needed to cause the anaphylactic reaction. If the antibody concentration were low, few sites would be sensitized, and a large number of antigen molecules would be required to cause the sensitized sites to react at the minimal rate. Exposure to very dilute antigen concentrations would cause desensitization without reaction because the rate of combination of the antigen with antibody would be too low. The inhibitory effect of very large antigen concentrations may be due to some non-specific action of the protein on the cells. Mongar and

Schild (146) have suggested that an enzyme system is activated by the antigen-antibody reaction. Perhaps the product of the initial enzyme reaction is a substrate for a second system, and if the product is not present in high enough concentration, subsequent steps leading to anaphylaxis may not occur. It remains to be seen what the exact role the antigen concentration plays in the anaphylactic reaction.

Time Course of Histamine Release.

The rate of appearance of histamine following exposure of sensitized tissues to antigen was studied. The velocity of histamine release was measured at 37.5, 32.4, and 27.3 C.

Methods.

The hearts from actively sensitized animals were removed without perfusion, and the atria were trimmed from the ventricles in a bath of oxygenated Chenoweth's at room temperature. The aortic stumps and a portion of the descending aorta were removed and put into warm oxygenated Chenoweth's solution. The ventricles were sliced about 0.8 mm thick. After the dissection procedure, the tissues were placed in Chenoweth's solution at 37.5 C. The tissues were divided into two groups. One group was challenged with antigen in Chenoweth's; the second was challenged with antigen in Chenoweth's containing 2 μg/ml of the histaminase inhibitor, semicarbazide. Three atria were challenged together in one container; similarly, three ventricles were challenged together. The aortic tissues were challenged only in the presence of semicarbazide. At the start of the challenge period, ovalbumin was added to make the

final concentration 0.5 mg/ml. Samples were removed at 2.0, 10, 20, and 60 minutes after antigen was added. A second experiment was performed; the sampling times were 0.5, 1.0, and 5.0 minutes after the start of the challenge procedure. Strips of the ileum from each guinea pig were tested for Schultz-Dale reactions; one of the twelve failed to react. After the last sample had been taken, the tissues were removed and dried to constant weight.

The sensitization of tissues for the second kind of time-course experiment was performed by perfusing the hearts of normal guinea pigs on the Anderson apparatus with 75 ml of Chenoweth's solution containing 150 μg/ml antibody. After the sensitization step, the hearts were perfused with 50 ml of antibody-free Chenoweth's. The atria were removed from the ventricles while the hearts were on the Anderson apparatus, and were placed in 3 ml of Chenoweth's solution. Antigen was added to make the solution concentration 0.5 mg/ml. After one minute, the tissues were transferred to a second container; successive transfers were made after the third, and fifth minutes. At the end of the seventh minute, the tissues were removed, blotted, and weighed. The containers of antigen solutions were assayed for histamine. The tissues were challenged at 37.5 C, 32.4 C, and 27.3 C.

Results and Discussion.

Histamine release begins shortly after sensitized tissues are exposed to antigen. Figures 6 and 7 show the time course of the appearance of histamine as it is released from challenged sensitized tissues. The reaction is essentially complete within ten minutes, and is half

FIGURE 6

TIME COURSE OF HISTAMINE RELEASE FROM
ACTIVELY SENSITIZED ATRIAL AND AORTIC TISSUE

TIME COURSE OF HISTAMINE RELEASE FROM ATRIAL AND AORTIC TISSUES

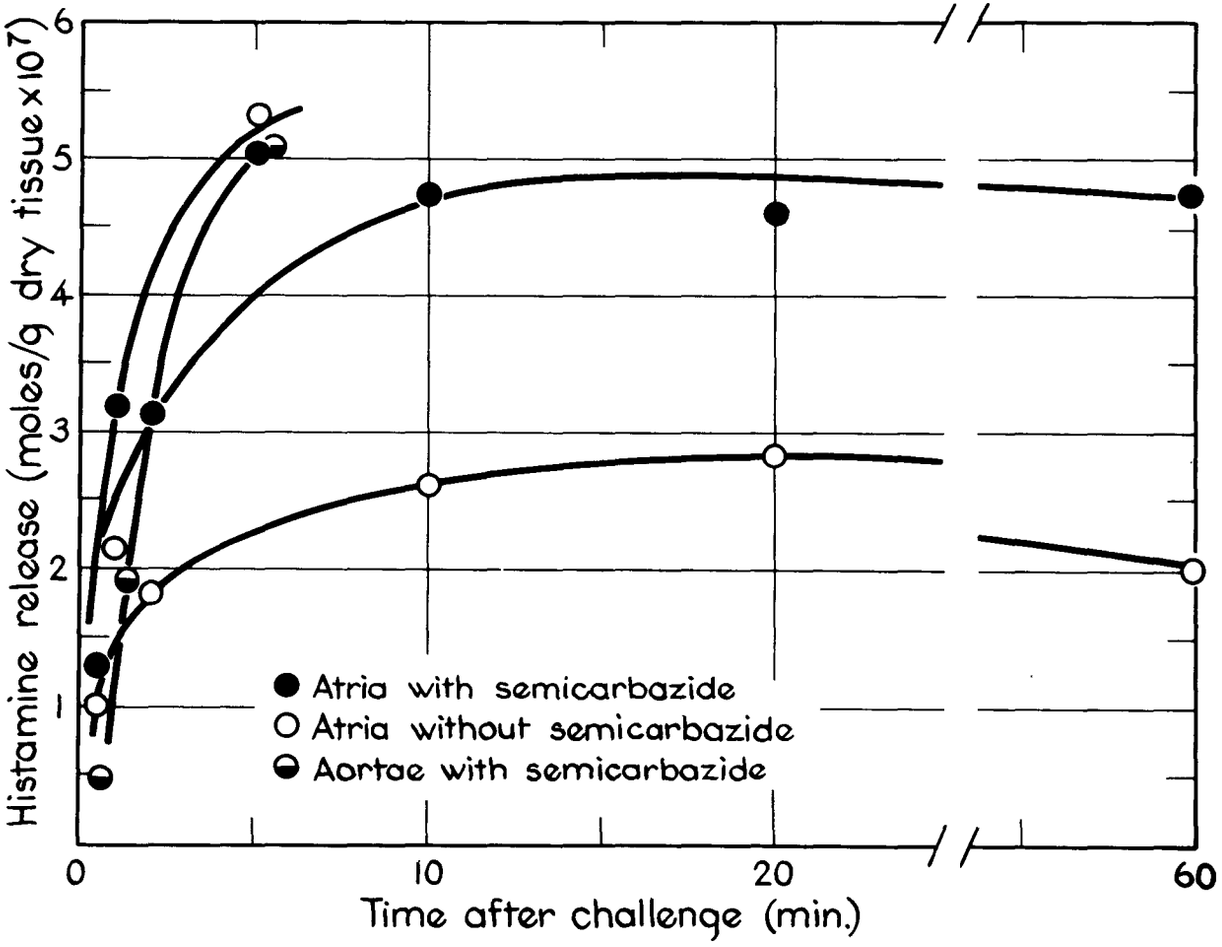
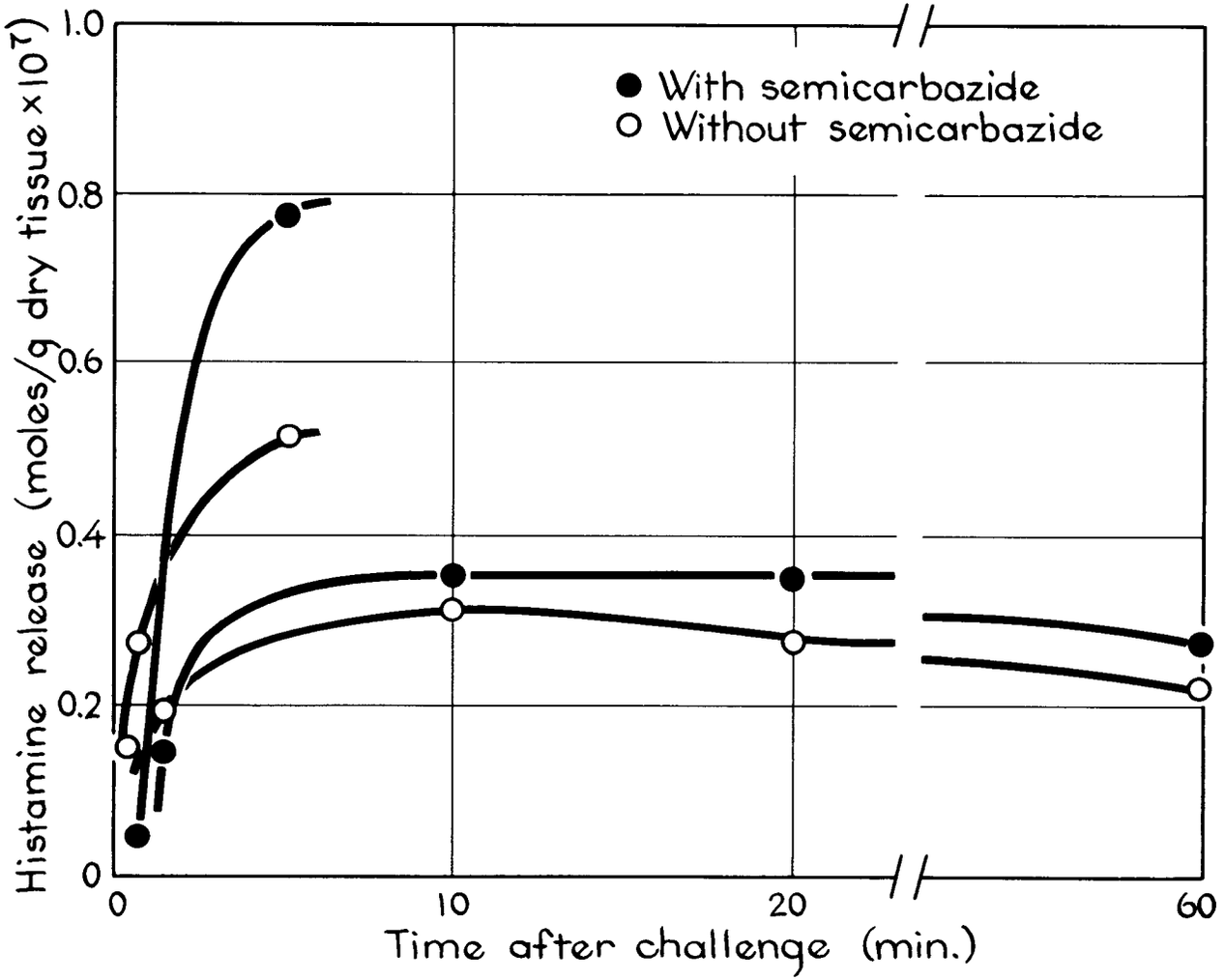


FIGURE 7

TIME COURSE OF HISTAMINE RELEASE FROM
ACTIVELY SENSITIZED VENTRICULAR TISSUE

TIME COURSE OF HISTAMINE RELEASE FROM VENTRICULAR TISSUE



complete within three minutes. The amount of histamine present in the incubation solution decreases slowly after ten minutes. The presence of semicarbazide (2 μg/ml) does not reduce the rate of disappearance. There does appear to be a difference in the magnitude of the total histamine released between the tissues challenged with and the tissues challenged without semicarbazide. Ileal strips of one of the 12 animals used, however, failed to give a Schultz-Dale reaction. Three hearts were used per experiment. If one of the ventricles and atria had not been sensitized, then the observed histamine release would have been 2/3 the release from a completely sensitized group. The curves for one of the experiments using atrial tissues and one of the experiments using ventricular tissues differ in the amount of histamine released by about the expected value. An alternative, but unlikely, explanation is that the anaphylactic reaction in the heart triggers a very active but short-lived histaminase.

The rapid release of histamine from sensitized guinea pig tissues is characteristic of the anaphylactic response. Chakravarty (42) reported that the release from sensitized lung was complete in 8 minutes, and that there seemed to be a histamine "destroying" factor which reduced the amount of histamine present in the incubation solution after 16 minutes; this factor was resistant to phenol. Similar release rates have been reported by Mongar and Schild (147) for guinea pig lung, uterine, and aortic tissues. Nielsen et al. (173) reported that the release of histamine from shocked guinea pig ileal strips reached a maximum value about five minutes after the start of the challenge procedure. They reported that the amount of histamine in the incubation

solution decreased after five minutes, but the loss was reduced substantially when the solution contained 2 μg/ml semicarbazide.

The effect of temperature on the rate of release of histamine from challenged atrial tissues is shown in Figure 8. There is a remarkable reduction in the rate of release at sub-normal temperatures. Temperature changes will affect the processes involved in the movement of antigen to sensitized sites and in the movement of histamine from the release sites to the incubation solution. These processes generally are considered to be diffusion controlled and should respond to temperature changes; the diffusion constant is directly proportional to temperature. A ten degree change around the normal temperature of 310 K would result in only about a 3 per cent change in the diffusion constant, and thus should contribute only a small fraction to the observed large temperature coefficient. To the extent that the effect of diffusion can be neglected, the initial velocities of histamine release can be used to calculate the overall activation energy of the histamine release process.

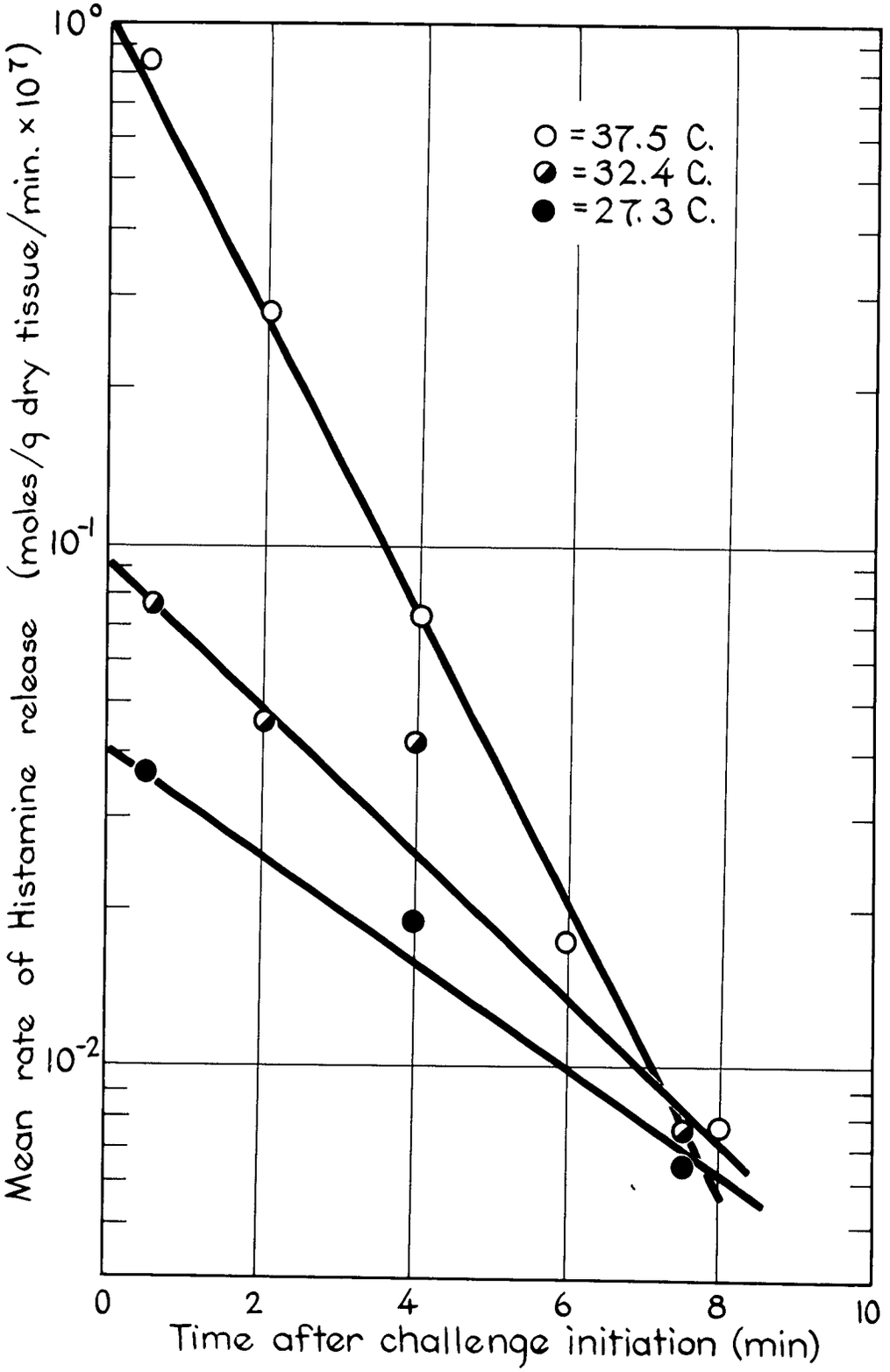
The curves of Figure 8 show that the overall histamine release phenomenon follows simple first order chemical kinetics; the logarithms of the observed velocities are related linearly to the duration of the reaction. The Arrhenius equation, relating the specific reaction rate to the absolute temperature can be integrated and rearranged to give the following relation between the activation energy and the rate constants at two temperatures:

$$\log \left(\frac{k_2}{k_1}\right) = \frac{H_a}{2.303R} \left(\frac{T_2 - T_1}{T_1 T_2}\right) \qquad [2]$$

FIGURE 8

RATE OF HISTAMINE RELEASE FROM IN VITRO
SENSITIZED ATRIA AS A FUNCTION OF TEMPERATURE

RATE OF HISTAMINE RELEASE AS A FUNCTION OF TEMPERATURE



where H_a = activation energy

R = universal gas constant

T_1 = absolute temperature at which the rate constant k_1 was measured

T_2 = absolute temperature at which the rate constant k_2 was measured.

The activation energy is the slope of the line relating the logarithm of the specific reaction rate to the reciprocal of the absolute temperature. Figure 9 is a plot of these values, obtained by extrapolation to zero time using the curves of Figure 8. The slope of the straight line fitted to the three points by the least squares weighting method corresponds to an activation energy of 28 kcal/mole. The effect of temperature on the anaphylactic reaction is complex. Mongar and Schild (150) reported that the Q_{10} (ratio of reaction rates, 10 C apart) for histamine release from lung tissue was 12 corresponding to an activation energy of 46 kcal/mole. Halpern et al. (82) reported a Q_{10} of 2.5 for the Schultz-Dale reaction over the range of 30 C to 38 C. The difference between the low value obtained for the Schultz-Dale and the high rates for lung and heart tissues probably is due to the inherent differences between the test systems. Mongar and Schild (150) suggested that the consequence of the antigen-antibody reaction was the activation of a short-lived enzyme system which had a high temperature coefficient.

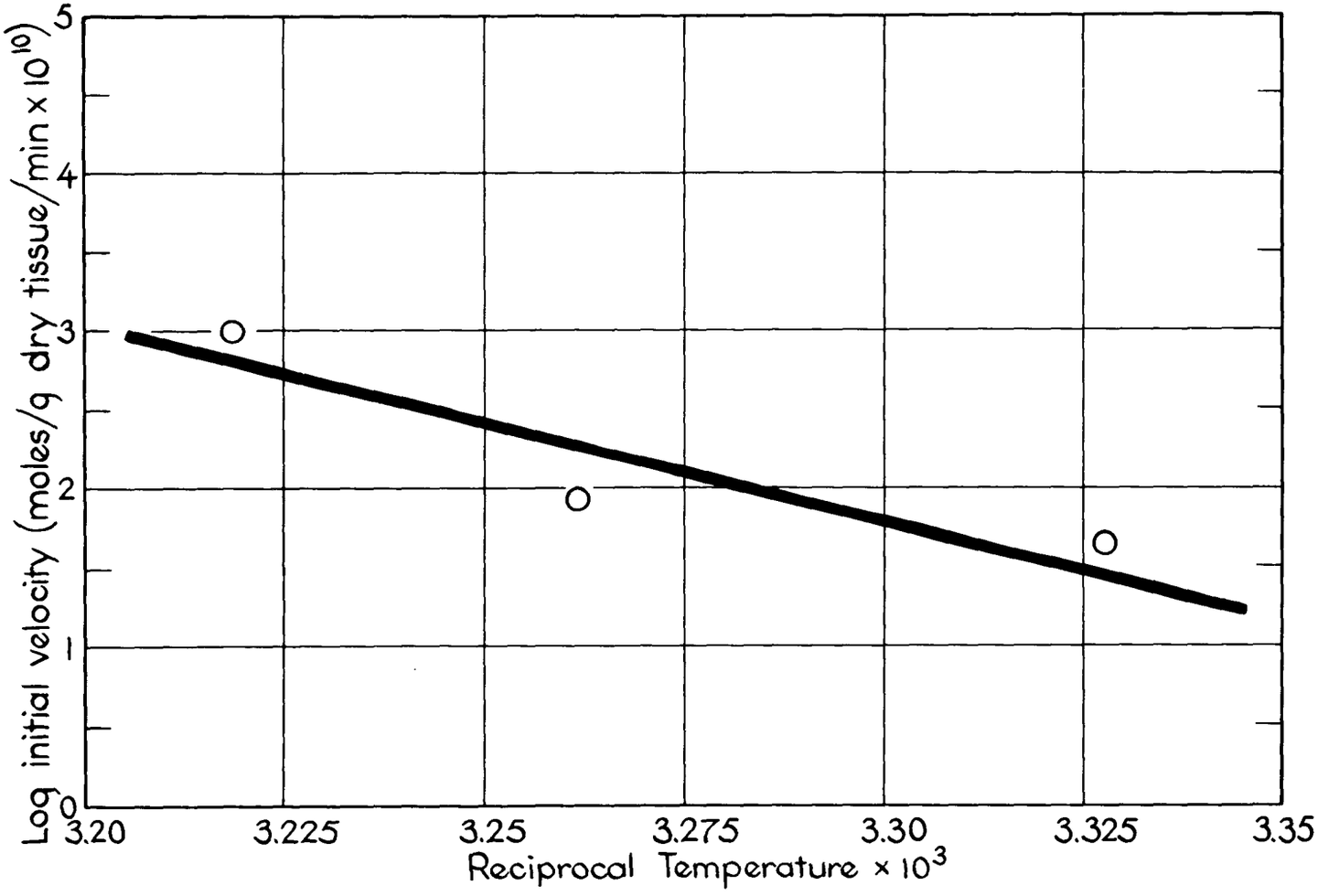
Histamine-destroying Factor in Heart Tissues.

The presence of an endogenous histamine destroying factor (histaminase) could mitigate the effects of anaphylactic shock on the heart.

FIGURE 9

INITIAL VELOCITY OF HISTAMINE RELEASE FROM
IN VITRO SENSITIZED ATRIA AS A FUNCTION OF TEMPERATURE

INITIAL VELOCITY OF HISTAMINE RELEASE AS A FUNCTION OF TEMPERATURE



Semicarbazide, a histaminase inhibitor, has been shown to potentiate the effects of histamine on isolated guinea pig ileal strips (154), and the addition of semicarbazide to the challenge solution increased the release of histamine from shocked ileal strips (173). Some tests were made with heart tissues to determine if their histaminase activity could be significant with respect to the amount of histamine released by them during shock.

Methods.

Normal guinea pig hearts were perfused on the Anderson apparatus with Chenoweth's solution. Histamine was added to the perfusing fluid to make the concentration comparable to the concentration found in the perfusate during the course of a typical maximal anaphylactic response. Four concentrations were used: 5×10^{-7} M, 1×10^{-6} M, 5×10^{-6} M, and 1×10^{-5} M. Semicarbazide was added to the perfusate to a concentration of 2 μg/ml for one group of hearts, and was omitted for a second. The perfusates were assayed for histamine.

In order to better localize any histaminase activity, the hearts of normal pigs were removed, and the atria, ventricles, and aortic stumps were separated. The ventricles were minced. The separated tissues were placed in containers of Chenoweth's with histamine and incubated at 37 C. Samples were withdrawn at zero time and intervals thereafter and assayed for histamine.

Results and Discussion.

The highest histaminase activity found was 4 μg of base per g of wet tissue per hour. This value is so low that the experimental technique

used to measure the activity does not permit any statements to be made about the effect of histamine concentration on the rate of destruction. The maximal rate of release of histamine from shocked hearts is at least 120 μg/g/hour, which is substantially greater than the rate of destruction. It does not seem likely that heart histaminase has much effect in reducing the intensity of the anaphylactic reaction of the heart. Lindell (134) measured the histaminase activity of various guinea pig tissues and found that the liver had the highest activity (22 μg/g/hr) and that the activity of the gut was 4.5 μg/g/hr. Nielsen et al. (173) reported that the gut releases about 6 μg/g/hr of histamine in the presence of semicarbazide. The histaminase activity of the gut does comprise a substantial fraction of the histamine release rate; gut histaminase activity, therefore, can affect the Schultz-Dale in contrast with the lack of effect on the anaphylactic reaction in heart tissue.

Successive Sensitization.

Isolated ileal strips have been sensitized at least five times in succession by Nielsen et al. (173). Successive Schultz-Dale responses declined in a regular fashion; the decline was attributed to the presence of non-specific γ-globulin in the antibody used. We made an analogous study, using guinea pig atria as a test system.

Methods.

The atria of six guinea pigs were prepared by trimming them from the ventricles under a stream of warm Chenoweth's solution. Two groups of three atria were incubated for one hour in 100 μg/ml antiovalbumin.

At the end of the incubation, the tissue groups were washed three times for five minutes each. After the last wash, they were transferred to a container of Chenoweth's and antigen (858 μg/ml). The reaction was permitted to proceed for 10 minutes. After the challenge step, the tissues were washed again three times, and reincubated in 100 μg/ml antibody for 1 hour. The incubation and challenge cycle was repeated for a total of five cycles. The challenge solutions were assayed fluorometrically for histamine, and the tissues were dried to constant weight after the last cycle.

Results and Discussion.

Histamine was released during the first sensitization-challenge cycle, but no additional release occurred during subsequent cycles. The total γ-globulin concentration was 500 μg/ml of which 20 per cent, or 100 μg/ml, was precipitating antiovalbumin. If histamine-releasing cells were to have only one sensitizable site, then the presence of four times as much γ-globulin as antibody would have prevented the sensitization of 80 per cent of the cells. Successive sensitization-challenge cycles would have released a fraction of the histamine which was in "protected" cells. In the first part of the experimental portion of this thesis it was shown that atrial tissue contains about $4.5 - 5 \times 10^{-7}$ moles of histamine per g of dry tissue. The amount released during the first cycle of this experiment amounts to at least 80 per cent of the maximum. These results conform to the possibility alluded to previously that histamine-releasing cells have several sensitive sites.

Nielsen and Feigen (173) reported that they were able to resensitize guinea pig ileal strips and produce several Schultz-Dale reactions. The magnitude of the reactions declined in a regular fashion: they found that the rate of decline was 26 per cent per cycle. The antibody they used contained 26 per cent precipitating antiovalbumin. This correlation suggested to them that the number of reactive sites that were sensitized was in proportion to the fraction of the precipitating antibody present. The amount of histamine released from a Schultz-Dale reaction represents a very small fraction of the total histamine present. The Schultz-Dale reaction site may have room for only one antibody molecule, so that the presence of non-specific γ-globulin will block sites in proportion to the amount of γ-globulin present. The reactive cells of heart tissue seem to have several sensitive sites.

Inhibition of the Anaphylactic Reaction by Homologous γ-Globulin.

Many investigators have observed and reported that the anaphylactic response of intact animals and isolated tissues can be reduced or inhibited if large doses of γ-globulin homologous with the antibody used for sensitization are given before challenge. Several theories have been suggested to explain the phenomenon, but the consensus is that the non-specific γ-globulin molecules interfere by blocking or occupying a site which could have been occupied by a sensitizing molecule.

It seems reasonable that some of the problems involved in the study of the sensitization phenomenon can be attacked with the information obtained from inhibition experiments. By using a pair of non-cross reacting antigen-antibody systems the phenomenon of interference can

be investigated, and the amount of antibody required to produce sensitization can be estimated.

Methods.

The atria from normal guinea pigs were removed from the ventricles while they were perfused with normal Chenoweth's solution. When three atria had been prepared, they were tied together, and incubated at 37 C in antibody solution. Three atria were used for each experimental point; the antibody concentrations and incubation times are listed in Table VIII. The incubation volume was 30 ml; the solution was changed at hourly intervals. After the incubation step, the tissues were washed three times, for 5 minutes each. They were challenged with ovalbumin; the reaction was permitted to proceed for 10 minutes. The tissues were washed again three times, and challenged for 10 minutes with BSA. The tissues were dried to constant weight, and the challenge solutions were assayed for histamine. The procedure is summarized in Table VIII.

The antibodies and antigens used are described in the Materials and Methods Section.

The possibility that the antiovalbumin would react with the BSA, and that the anti-BSA would react with ovalbumin was tested by sensitizing sets of tissues with each antibody, and challenging with the "wrong" antigen.

Results and Discussion.

Table IX shows that BSA does not release histamine from tissue sensitized with ovalbumin, and ovalbumin does not release histamine

TABLE IX

HISTAMINE RELEASE FROM ATRIAL TISSUES SENSITIZED WITH ANTIOVALBUMIN AND ANTI-BSA

Antibody	Antigen	Histamine Release moles/g dry tissue x 10^7
Antiovalbumin	Ovalbumin	3.60
Antiovalbumin	BSA	0.00
Anti-BSA	BSA	3.76
Anti-BSA	Ovalbumin	0.00
Incubation Solution		0.00

Antiovalbumin concentration = 100 μg/ml
Anti-BSA concentration = 121 μg/ml
Antigen challenge concentration = 100 μg/ml
Time of incubation = 1 hour
Time of challenge = 10 min
Temperature for incubation and challenge = 37 C

TABLE VIII

EXPERIMENTAL POINTS AND HISTAMINE RELEASED FROM ATRIAL TISSUES SENSITIZED WITH MIXED ANTIBODIES

Precipitating Antiovalbumin per cent	Precipitating Anti-BSA	Incubation Time Hours	Histamine Release moles/g dry tissue x 10^7 γ-globulin concentration			
			100 μg/ml	20 μg/ml	5 μg/ml	1 μg/ml
1.0	15.6	2	0.0			
		3	0.03			
2.0	14.8	2	0.087			
		4			0.089	
3.0	14.0	3		0.042*		
4.0	13.2	3			0.127*	
5.0	12.4	2	1.95*			
		3		0.623*	1.11*	0.084*
		5				0.04
7.0	10.7	3		1.32*	1.65*	0.116*
10.0	8.3	2	4.62*			
		3		5.24*	2.31*	0.93*
		5				0.81
20.0	0.0	2	4.41*	3.33		
		3		4.88*	3.64*	3.91*
		5				2.89

Incubation, wash, and challenge volumes were 30, 35, and 25 ml, respectively. [ovalbumin] = 1 mg/ml. Temperature was 37 C and challenge time 10 min.

*points used in analytical treatment

from tissue sensitized with ovalbumin, and ovalbumin does not release histamine from tissues sensitized with anti-BSA. There was no detectable release of active material into the incubation solution during the sensitization procedure. Therefore, as far as this system is concerned, the two antigen-antibody systems are inert to each other, and any histamine release by, for example, ovalbumin, is due to the presence of histamine releasing sites sensitized with antiovalbumin.

Weigle (232) found that rabbit anti-BSA did not react with either guinea pig albumin or non-mammalian albumins. The results of the tissue sensitization study agree with Weigle's observations of the gel diffusion system.

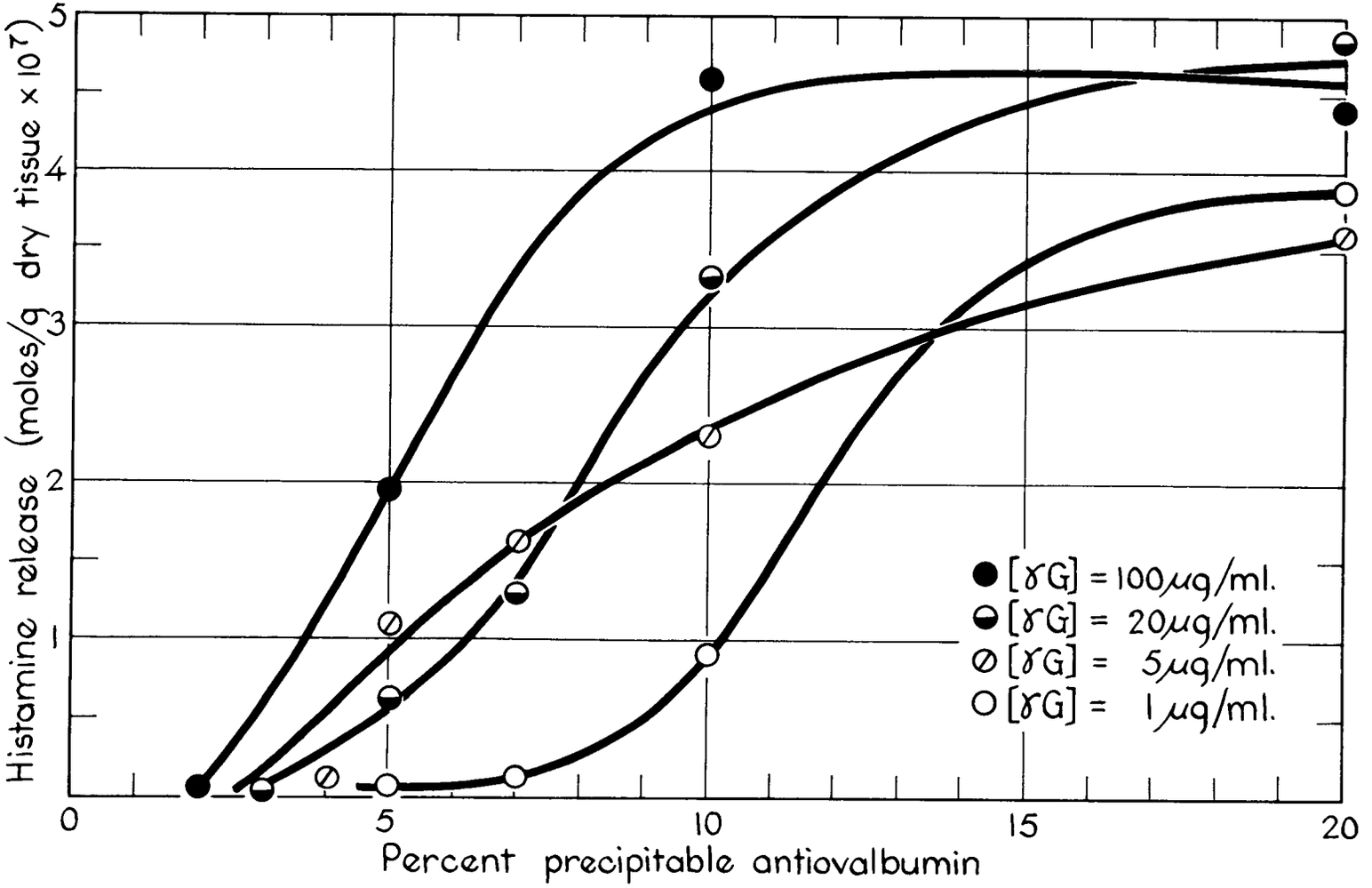
Figure 10 shows the effect of reducing the antiovalbumin content of the incubation solution on histamine release resulting from challenge with ovalbumin. The data obtained from the challenge with BSA will be discussed in the next section. The sigmoid curves show that as the fraction of the γ-globulin comprised by antibody is reduced, the amount of histamine released is reduced. Dilution of the antibody with γ-globulin is more effective in reducing histamine release than is diluting the antibody solution with Chenoweth's, i.e., reducing the total γ-globulin concentration. The curvature of the lines make it difficult to express precisely the effect of non-specific γ-globulin on the sensitization process. It can be said that about a hundred-fold increase in γ-globulin concentration is needed to offset a 50 per cent dilution of the antibody with non-specific γ-globulin.

The sigmoidal curve is representative of many biological phenomena. Many biological systems are made up of an ensemble of similar units,

FIGURE 10

HISTAMINE RELEASE FROM *IN VITRO* SENSITIZED ATRIA
AS A FUNCTION OF PRECIPITATING ANTIOVALBUMIN CONTENT

HISTAMINE RELEASE AS A FUNCTION OF PRECIPITATING ANTIOVALBUMIN CONTENT



whose sensitivity to the independent variable may vary among individual units. The total response is representative of the statistics of the ensemble. A cumulative probability distribution function is very similar in shape to biological response curves. However, the probability curve is somewhat unwieldy to handle and a simpler treatment is used.

The von Krogh transform [3] often is used.

$$\log x = \log k + 1/n \log y/1 - y \qquad [3]$$

where x = independent variable

y = response/maximum response

k, n are system parameters.

This transformation was introduced by von Krogh (121) to simplify the treatment of dose-response curves. The slope parameter, n, has been interpreted as being related to the standard deviation of the probability function or as being related to the heterogeneity of the system. The parameter k is the value of the independent variable which is associated with a half-maximal response. The von Krogh transform has been used successfully to treat the response of guinea pig ileal strips to histamine (66) and to quantitate the titration of hemolytic complement (109).

The von Krogh transform was applied to the data of Figure 10; 5×10^{-7} moles per g dry tissue was chosen as the maximal response. The results of the transformation have been plotted in Figure 11 and the n and k values are listed in Table X. The latter were calculated by the method of least squares. The values for the per cent precipitating

FIGURE 11

VON KROGH TRANSFORMATION OF THE DATA OF FIGURE 10

VON KROGH TRANSFORMATION OF THE DATA OF FIGURE 10

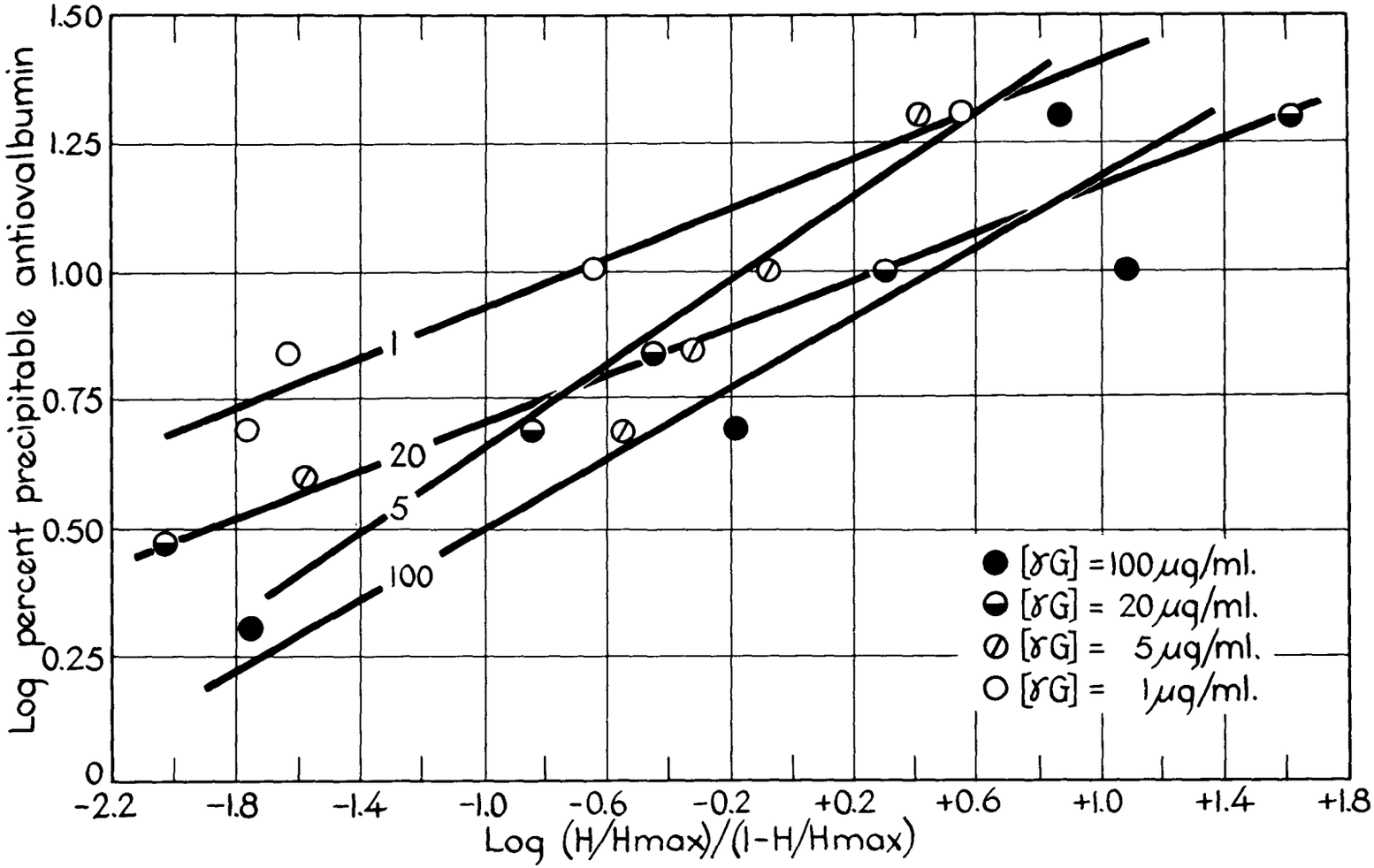


TABLE X

VON KROGH PARAMETERS OF THE LINEARIZED DATA OF THE INHIBITION EXPERIMENT

Bulk Phase γ-globulin Concentration (μg/ml)	Slope (1/n)	Intercept k	Per Cent Precipitating Antiovalbumin
100	0.352	0.825	6.68
20	0.230	0.930	8.51
5	0.402	1.037	10.89
1	0.239	1.171	14.83

antibody required to produce half maximal sensitization increase as the bulk phase concentration of γ-globulin decreases. These values can be used to calculate a relation between bulk phase γ-globulin concentration and the per cent precipitating antibody required to produce half-maximal sensitization. The relation, calculated by the method of least squares, is

$$\log BF = 6.92 - 5.96 \log P \qquad [4]$$

where BF is the bulk phase γ-globulin concentration in μg/ml, and P is the per cent precipitating antibody in the incubation solution.

If the expression is used to estimate the bulk phase concentration needed with pure precipitating antibody, then the value obtained is 10^{-11} g/ml.

The conditions which would be required to achieve sensitization in the presence of 10^{-11} g/ml of antibody probably would include very thorough removal of non-antibody γ-globulin and a very long incubation period. These conditions may be impossible to achieve in practice, but Binaghi et al. (22) were able to sensitize passively guinea pig ileal strips using 10^{-8} g/ml of antibody which was not prepurified by specific precipitation. If the results of the earlier experiments using radioactive antibody can be extrapolated to the 10^{-11} g/ml range, then about 5×10^{-12} g or 2×10^{7} molecules would be retained per g of dry tissue. This number of molecules is small compared with the total number of cells in a gram of dried atrial tissue so that it seems that sensitization and the anaphylactic release of histamine is a very efficient process. These extrapolations may be unjustified for

concentrations much outside the range used in this experiment, but the value of 10^{-11} g/ml certainly seems reasonable as a lower limit and may not be more than one or two orders of magnitude below the "real" value.

The source of the histamine released during anaphylactic shock generally is presumed to be the mast cells in the connective tissue. Histamine is released from tissues rich in mast cells when the tissues are treated to cause mast cell degranulation. Mast cells from sensitized tissues degranulate when they are treated with antigen. Riley (197) estimated the histamine content of mast cells contained about 2×10^{-11} g of the base or 2×10^{-13} moles per cell. Riley's value is used in the following discussion to estimate the mast cell population of guinea pig atrial tissue. The atria release all of their histamine when they are challenged; the maximal release value is about 5×10^{-7} moles per g of dry tissue. If the histamine comes only from mast cells, then there would be about 2.5×10^{6} mast cells per g of dry tissue. Padawer (183) estimated the volume of a mast cell to be 1.4×10^{-9} cc. Mast cells have a high ash content; the dry-to-wet weight ratio might be 1:4 and the density about 1.2. The dry weight of a single mast cell would be about 4×10^{-10} g ; the weight of 2.5×10^{6} mast cells would be 1 mg, corresponding to 0.1 per cent of the dry weight of the atrial tissue.

There are no data available concerning selective uptake of γ-globulin molecules by mast cells. Humphrey and Mota (92) inspected the distribution of fluorescence-tagged antibody among the cells in guinea pig mesentary tissues and they were unable to find any evidence of selective

adsorption of tagged antibody. There are no data available about the distribution of antibody between the extracellular fluid and cell surfaces; nor are there any data about the relative surface area of the mast cell population with respect to the total area of cell surface available for antibody binding. In the absence of contrary evidence, it is suggested that the mast cells adsorb antibody in proportion to the mast cell population; if 0.1 per cent of the tissue is comprised of mast cells, then 0.1 per cent of the antibody retained by the tissues is adsorbed to the mast cells. The lack of data and the assumptions made to supply the necessary starting points make hazardous a literal interpretations of the following discussion. The results will be subject to substantial revision when data become available to replace the assumptions.

The preceding discussion forms the basis for estimating the distribution of antibody molecules on mast cells. The number of molecules probably will be distributed randomly among the cells.

There are several ways to estimate the statistics of a random population. Following the notation of Wilkes (238), binomial distribution function,

$$f_B(x) = \frac{n!}{x!(n-x)!} \; p^x(1-p)^{n-x}, \qquad [5]$$

can be used to calculate the probability, p, that exactly x molecules of antibody would be bound to a cell with a capacity of n independent antibody binding sites. The binomial distribution function is tedious to use when n is large and p is small. The Poisson distribution function,

$$f_P(x) = \frac{(m)^x e^{-m}}{x!} \quad \text{where} \quad m = np \ , \qquad [6]$$

is more useful in this case. Both $f_B(x)$ and $f_P(x)$ are used for integral values of x. If the distribution of a random continuous variable is desired, then the normal or Gaussian distribution is used.

$$f_N(x) = \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad \text{where } \sigma \text{ is} \qquad [7]$$

the standard deviation and μ is the mean value.

Statistical analysis has been used to investigate the mechanism of red cell lysis by complement. Alberty and Baldwin (2) used the binomial distribution function to calculate the number of substrate sites, r, out of a total of m sites that were required to be acted on by complement for lysis to occur. In order for the predicted lysis curves to match with the observed data, it was necessary to choose r to be about ten. The sigmoidal lysis curve could be predicted on the basis of purely statistical techniques. Mayer (109) has summarized the current conceptions of lysis and has shown that there is kinetic evidence that lysis is not a cumulative or multi-hit process. However, Alberty and Baldwin's calculations are useful in demonstrating how a purely statistical treatment can be applied to biological systems.

The present problem is to try to discover a schema which accounts for the observation that the degree of sensitization depends greatly on the fractional amount of precipitating antibody present in the incubation solution and depends to a very much lesser extent on the total γ-globulin concentration. If there were a minimum number of sites

on a mast cell which were required to have antibody molecules before sensitization occurred, then one would expect that this number would be the same for any cell sensitized in the presence of any reasonable concentration of antibody. The product of the number of sensitizing molecules per cell with a "correction factor" associated with the conditions of sensitization should be a constant. The "correction factor" may be accounted for by the results of purely statistical distribution of molecules over the cell surface.

The Poisson distribution function will be used to calculate the distribution of antibody molecules on mast cells. Before the calculation can be made, some additional extrapolations and assumptions must be made. The results of the radioactive antibody binging studies indicate that about 0.5 μg of antibody is bound per g of dry tissue per μg antibody per ml of the incubation solution. For the γ-globulin concentrations of 1, 5, 20, and 100 μg/ml, the amount bound would be 0.5, 2.5, 10, and 50 μg/g of dry tissue. The molecular weights of rabbit γ-globulin is about 1.6×10^5; the number of molecules bound per g of dry tissue would be 1.9×10^{12}, 9.4×10^{12}, 3.8×10^{13}, and 1.9×10^{14} molecules respectively. If 0.1 per cent of the molecules were bound to mast cells and if the number of mast cells per g of dry tissue were 2.5×10^6, then the number per mast cell would be 7.6×10^2, 3.8×10^3, 1.5×10^4, and 7.6×10^4. The area of a mast cell is about 6.5×10^{10} A^2 (183). If only about one tenth of the area were available for binding, then the area over which the binding could occur would be 6.5×10^9 A^2. The projected area of a rabbit γ-globulin molecule is about 1.2×10^4 A^2 ; the amount of antibody bound

in the presence of 100 μg/ml in the incubation solution would amount to about 14 per cent of the available area.

Let us suppose, further, that the formation of an Ag_2Ab complex on the cell surface is part of the histamine releasing mechanism. If this complex were stretched out, it would be about 600A long. If a non-antibody γ-globulin molecule were within about 600A of an antibody molecule, it might interfere with the formation of a suitable complex when antigen was present. Let us call a "Nominal Area" (NA) a 600A diameter region on the surface of the mast cell. The presence of more than one antibody or γ-globulin molecule in an NA renders that site insensitive. The area of an NA is about 2.8×10^5 A^2; there would be about 2.3×10^4 NA's distributed over the available area of a mast cell.

The Poisson distirubtion function, [6] can be used to calculate the number of NA's with 0, 1, 2 ... molecules within an NA. The number of NA's with only one antibody molecule and no non-specific molecules is calculated by multiplying the probability that an NA has one antibody by the probability that it has no non-specific molecules and taking the resulting product and multiplying it by the number of NA's per cell. The results of the calculation are shown in the last column of Table XI. The numbers in the last column are not identical, but they are reasonably close, being from 110-670. In the presence of "high" concentration of γ-globulin, substantial interference occurs, but at the lower concentrations, the molecules bound are spread thinly so that little interference occurs. If the table had been extended to less than 1 μg/ml, the mathematical model would

TABLE XI

CALCULATED DISTRIBUTION OF ANTIBODY MOLECULES ON IN VITRO SENSITIZED CELLS

[γ-globulin] μg/ml	No. of Molecules Per Cell	Fraction of PAOv Required for Half Sensitization Per Cent	PAOv Molecules Per NA	Non-specific Molecules Per NA	X	$f_p(x)$ PAOv	$f_p(x)$ Non-specific	No. of NA's with one PAOv and no Non-spec. Molecules
100	7.6×10^4	6.7	2.21×10^{-1}	3.08	0	8.02×10^{-1}	4.60×10^{-2}	1.9×10^2
					1	1.77×10^{-1}	1.42×10^{-1}	
					2	1.96×10^{-2}	2.18×10^{-1}	
20	1.5×10^4	8.5	5.53×10^{-2}	5.90×10^{-1}	0	9.46×10^{-1}	5.54×10^{-1}	6.7×10^{-2}
					1	5.23×10^{-2}	3.28×10^{-1}	
					2	1.45×10^{-3}	9.68×10^{-2}	
5	3.8×10^3	10.9	1.80×10^{-2}	1.47×10^{-1}	0	9.82×10^{-1}	8.67×10^{-1}	3.5×10^2
					1	1.77×10^{-2}	1.27×10^{-1}	
					2	1.59×10^{-4}		
1	7.6×10^2	14.8	4.89×10^{-3}	2.81×10^{-2}	0	9.95×10^{-1}	9.72×10^{-1}	1.1×10^2
					1	4.87×10^{-3}	2.73×10^{-2}	
					2	1.19×10^{-6}		

PAOv = Precipitating antiovalbumin

have predicted that the number of NA's with isolated antibody molecules would be essentially equal to the number of molecules bound to the cell. The probability that two or more antibody molecules occupy the same NA is very small; this suggests that if antibodies were distributed randomly, there is little chance that the trigger complex contains two or more antibody molecules. The results of the foregoing calculation are useful to support the suggestions that the effect of non-specific γ-globulin is to occupy sensitizable sites and that inhibition is the result of the presence of molecules which hinder the combination of antigen with antibody.

Consideration of the effect of antigen concentration on the release of histamine from sensitized cells was not included in the preceding discussion. The results of the experiments on the effect of antigen concentration suggest that cells which would not release histamine in the presence of dilute antigen solutions would release histamine if strong antigen solutions were used. This effect may have exaggerated the effect of changing the γ-globulin concentration. However, the results could be reinterpreted as being indicative of the number of sensitized sites required for histamine release in the presence of a given antigen concentration; the latter would influence the rate at which sites were triggered. Since the effect of raising the antigen concentration used to challenge the tissues is to increase the histamine release, the estimate of several hundred sites per cell is probably high.

The estimate of the number of antibody molecules required to sensitize a mast cell is based on several assumptions. Until more

information becomes available to substantiate or revise the assumptions, the accuracy of the estimate is at best an order-of-magnitude approximation. The estimated number probably is, based on the preceding discussion, between 10 and 200.

Double Sensitization.

Methods.

The γ-globulin diluent used in the inhibition experiment was rabbit anti-BSA. After the tissues were challenged with ovalbumin, they were washed three times in normal Chenoweth's and then challenged again with 0.3 mg BSA per ml of Chenoweth's. Some of the tissues released additional histamine.

Results and Discussion.

Figure 12 shows the sum of the histamine released by challenge with ovalbumin and BSA. The curves show a pronounced dip in the intermediate range of precipitating antibody concentration. These results suggest that sensitization of the tissues by anti-BSA was inhibited by the presence of antiovalbumin.

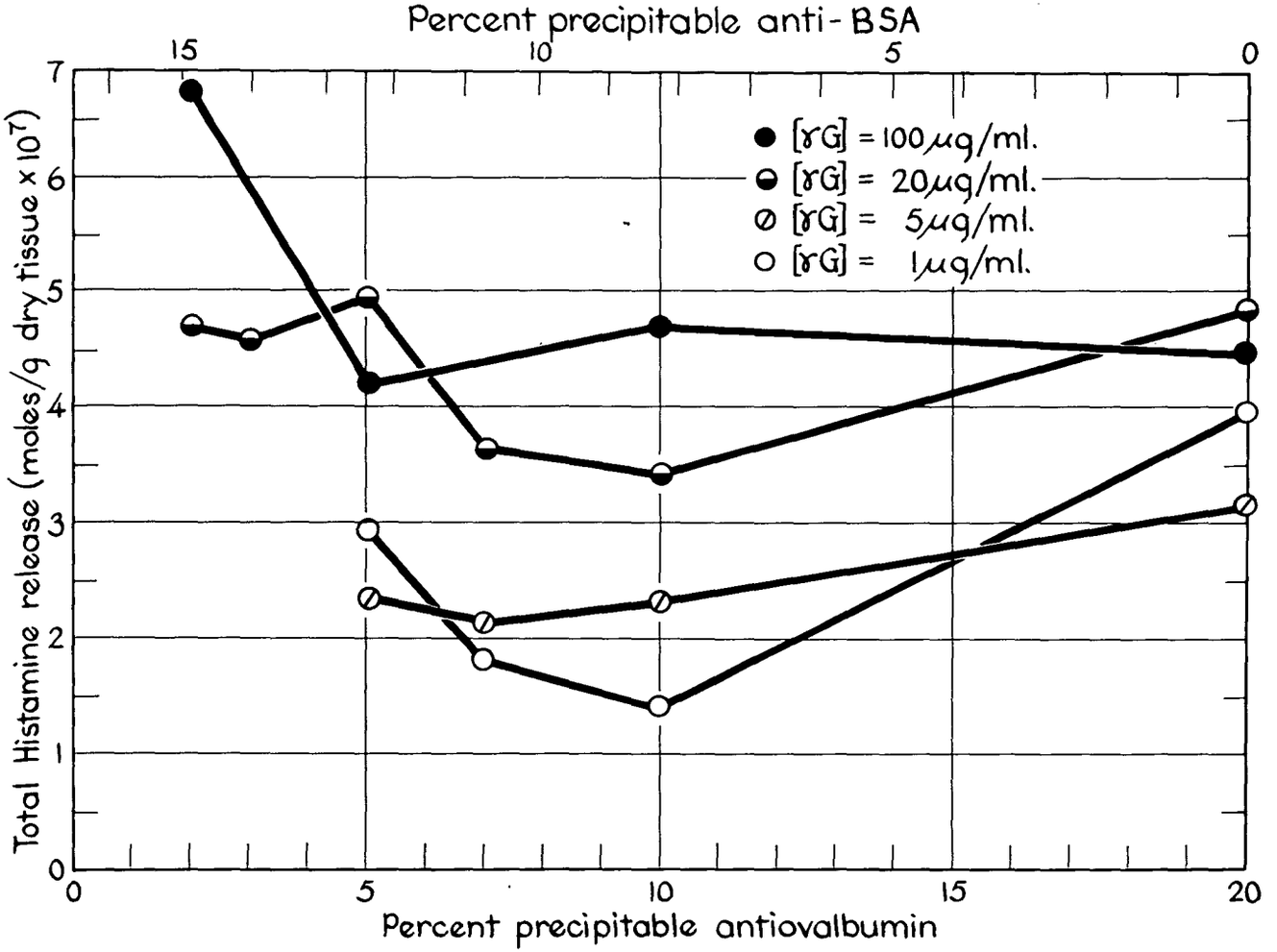
The curves of Figure 10 can be interpreted as being representative of the probability of sensitization of a histamine releasing cell. Therefore the curves can be used to estimate the probability of sensitization by anti-BSA as a function of its concentration. The functions used were the von Krogh transforms of the ovalbumin release data.

If a histamine releasing cell has many sites which can be

FIGURE 12

TOTAL HISTAMINE RELEASE FROM ATRIAL TISSUES SENSITIZED IN VITRO WITH TWO ANTIBODIES

TOTAL HISTAMINE RELEASED FROM ATRIAL TISSUES SENSITIZED WITH MIXED ANTIBODIES



sensitized, and if only a few of the sites are required to be sensitized if the cell is to be considered sensitized, then sensitization of a cell in the presence of two antibodies with different specificities may occur in one of the three following ways: the cell may not be sensitized to either antibody; the cell may be sensitized to one or the other; the cell may be sensitized to both. Some cells in the second category are included in the third category. The expression for the probability of sensitization may be written formally:

$$P_T = P_O + P_B - P_O P_B \qquad [8]$$

where P_T = Probability of sensitization by either antiovalbumin, anti-BSA, or both

P_O = Probability of sensitization by antiovalbumin

P_B = Probability of sensitization by anti-BSA

The values for P_O and P_B were calculation from the observed histamine release data plotted in Figures 10 and 11.

The calculations are summarized in Table XII. The absolute magnitudes of the calculated and observed values for total histamine release are not identical, but there is a definite parallelism. The correlation supports the suggestions that the degree of sensitization by an antibody is related to the precipitating fraction of the antibody and that histamine releasing cells have several sensitizable sites. The calculations also suggest that histamine release is not due to cumulative damage to reactive cells. Cells sensitized partially to both antigens would release histamine during the second challenge if histamine release

TABLE XII

CALCULATED PROBABILITY OF SENSITIZATION OF ATRIAL TISSUES SENSITIZED IN VITRO WITH TWO ANTIBODIES

[γ-globulin]	Precipitating Content		Probability of Sensitization		Total Probability	Histamine Release	
μg/ml	Anti-ovalbumin per cent	Anti-BSA	Anti-ovalbumin	Anti-BSA		Expected moles/g dry tissue x 10^7	Observed
100	10	8.3	.759	.642	.914	4.57	4.72
	5	12.4	.307	.852	.897	4.49	4.20
	2	14.8	.249	.905	.929	4.65	6.82
20	10	8.3	.668	.460	.821	4.11	3.43
	7	10.7	.349	.730	.828	4.12	3.64
	5	12.4	.0901	.837	.852	4.26	4.98
5	10	8.3	.415	.304	.593	2.97	2.31
	7	10.7	.230	.455	.580	2.90	2.12
	5	12.4	.116	.550	.602	3.01	2.38
	4	13.2	.0711	.581	.611	3.06	2.73
1	10	8.3	.1609	.077	.226	1.13	1.35
	7	10.7	.0414	.203	.236	1.18	1.82
	5	12.4	.0105	.320	.327	1.69	2.99

were the result of cumulative damage. There would be no dip in the total release curve. There is a dip in the curve, so that cumulative subthreshold damage does not seem to cause histamine release.

SUMMARY

The results of the experiments described in this thesis have suggested possible mechanisms for the process of passive sensitization and the mode of the action of antigen on sensitized tissue.

The sensitization process can be separated from the process of antibody uptake. The amount of antibody retained by in vitro sensitized tissues is proportional to the concentration of antibody present in the bulk phase of the solution used for sensitization. Temperature has little effect on the amount of antibody retained by the tissue as a whole but does affect the degree of sensitization achieved. The velocity of sensitization is proportional to the bulk phase concentration of antibody used. Sensitization seems to be associated with the attachment of antibody molecules to very specific locations on sensitizable cells. This attachement process may involve the dissociation of antibody molecules from loose bonds with non-reactive cells and transfer to reactive cells. Experimental results have been presented which show that sensitization is related to the amount of precipitating antibody present in a mixture of antibody and homologous γ-globulin. In the case of guinea pig atrial tissue, antibody probably is attached to several sites on reactive, histamine-releasing, cells. The minimal number of sites on reactive cells has been estimated to be between ten and 200.

The presence of homologous γ-globulin in the sensitizing solution can reduce the degree of sensitization achieved. This inhibition process seems to result from the binding of non-specific molecules too close

to antibody molecules so that subsequent reaction of the antibody with antigen is hindered sterically.

The treatment of sensitized guinea pig heart tissues with antigen results in the release of histamine. The combination of antigen with antibody seems to be the trigger for the histamine releasing process. Histamine is released under conditions which suggest that there is a threshold rate of triggering of sensitized sites on reactive cells. If the rate of triggering is below the threshold, desensitization without histamine release may occur. When doubly sensitized tissues were desensitized to one antigen and challenged with the second antigen, the amount of histamine release observed suggested that histamine release is not the result of cumulative damage. Histamine release appears to result from the triggering of a minimal number of sensitized sites within a certain relatively short time.

The experiments in this thesis suggest some of the mechanisms of part of the phenomenon of anaphylaxis. Much work remains to be done before the whole process is described completely.

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