POSSIBLE MECHANISMS OF HISTAMINE-HUMAN GAMMA GLOBULIN INTERACTIONS

by

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INTRODUCTION

Theories of Allergy

The strange phenomonen of anaphylaxis, which means increased susceptibility, has been the subject of intense research during the twentieth century. Increased emphasis was added by Meltzer (16) who first called attention to the similarities existing between the condition of the lungs of guinea pigs that died of anaphylactic shock and those of patients suffering from asthma. These observations were confirmed for many symptoms of both humans and animals until gradually the two processes, anaphylaxis and allergy, became more nearly one.

Among the theories of the mechanism of anaphylaxis two important ones have been advanced to explain this phenomonen of anaphylaxis. The anaphylatoxin or humoral theory postulates a union of the antigen or foreign protein with its specific antibody resulting in a cleavage of the antigen with the subsequent release of a poisonous substance. It is this poison which in turn causes the shock or allergic reaction in certain organs.

Friedberger (6, 7) was one of the first to advance this theory. He was able to isolate a poisonous substance from the precipitate of an antigen-antibody reaction which produced symptoms very similar to anaphylaxis in the guinea pig.

The cellular theory, as opposed to the humoral theory, explained the phenomonen on the basis of cell disturbance in the absence of any poison. When the foreign protein is introduced, local tissue cells are called upon to dispose of it. During the

incubation period these cells are constantly under stress to digest the protein and when the shocking dose of protein is administered the increased functionality of the cell is overwhelmed and the tissue undergoes shock. While the cellular mechanism is purely speculative the site of reaction has supporting evidence. Dale (3) isolated the uterus of a sensitized guinea pig and after washing it free of body fluids suspended it in a salt solution and observed the similar type of anaphylactic shock when the sensitizing protein was administered. Similar experiments by Pearce and Eisenbrey (21) on other laboratory animals have confirmed this.

Role of Histamine

Histamine is the common name given to 4 (or 5) ethylamineimidazole. Histamine has long been identified as the poisonous substance referred to in the humoral theory. Many similarities have been shown to exist both in the symptoms and the physiology of anaphylaxis with those produced by direct administration of histamine (4). The isolation of histamine from the lung tissue of an ox which had undergone anaphylaxis by Best, et al (2) provided good evidence that this was indeed the toxic substance which caused anaphylaxis. They were also able to isolate it in quantities adequate to account for such activities.

More recently experiments have been directed towards elucidating the mechanism by which histamine exerts its powerful physiological effect. Parrot, et al, (19 - 20) have reported success in their attempts to bind histamine in vitro to animal proteins. It

was found that the human blood plasma proteins, in particular gamma globulin, from normal individuals had the ability to bind histamine while the blood proteins from asthmatic patients did not bind histamine. Also these authors have been successful in binding histamine in vitro to the blood serum of unsensitized rats. Mickel (17) has reported that histamine is bound by bovine serum albumin.

Conversely, Kaplan and Davis (9) using carbon-14 labeled histamine could find no evidence of binding in vitro to the human plasma proteins by histamine. They also reported no binding of histamine to a homogenate of guinea pig tissue.

The purpose of this work was to further elucidate the mechanism by which histamine exerts its powerful physiological effect and to attempt to resolve the above mentioned discrepancies.

ANALYTICAL

Biological Methods

Biological methods were first used to detect the presence of histamine. These methods were the direct result of the observations of Dale and Laidlow (5) that histamine causes the constriction of various smooth muscles and that the muscle response is directly related to the amount of histamine present. Quantitative estimations of histamine were made by attaching the ileum separated from a guinea pig to a recording drum and measuring the distortion of the ileum caused by succeedingly lower concentrations

of histamine. The histamine is contained in a Tyrode solution (19) which surrounds the ileum. A single ileum may be used several times by washing free the histamine after each test. This method has been valuable in measuring antagonistic effects of certain antihistamines towards histamine. Staub (23) has correlated the efficiency of many antihistamine compounds by observing the inhibiting properties of these compounds towards the contraction on the guinea pig ileum caused by the action of standard histamine solutions.

Another biological method for the analysis of histamine has utilized the increased capillary permeability caused in animal systems by the administration of histamine. It is particularly useful in studying these systems in vivo. A dye, trypan blue, is injected into the organism and the increased rate of its infiltration, due to the presence of histamine, is compared to the rate of infiltration in a similar organism in the absence of histamine (Ramsdell, 22).

These biological methods for the determination of histamine seem to suffer from the disadvantage of being non-specific for histamine. Such physiologically active substances as choline and acetylcholine also cause such changes and therefore must be absent. Another disadvantage is that many laboratory animals must be sacrificed in the routine analysis.

Chemical Methods

The chemical methods for the determination of histamine had their origin in the work of Koessler and Hanke (14) who first demonstrated that histamine would couple with diazotized sulfonillic

acid to form a highly colored compound. In this method an alkaline solution of histamine is allowed to react with the diazonium salt and the color which is formed is compared with a standard. This method, although widely used, has suffered from the fact that the color bloomed slowly and began to fade after it reached its maximum intensity. This disadvantage has been, to a large extent, overcome in this work by extracting the colored product of the reaction of histamine and diazotized p-bromoanaline with n-butyl alcohol. This colored extract was found to be stable for two hours.

Other chemical methods for the analysis of histamine have been suggested. These include a gravimetric precipitation by 3,6-dinitro-2,5-dihydroxyquinone, reaction with 2,4-dinitrofluorobenzene and reaction with cobalt nitrate as proposed by Zimmerman (24). In the latter method it has been possible to distinguish between histamine and histidine since histidine does not form a color in vacuo with alkaline cobalt nitrate.

EQUILIBRIUM DIALYSIS

Theory

The study of the interaction between protein molecules and small ions in solution has been greatly facilitated by the method of equilibrium dialysis. In this method the large protein molecule is enclosed in a semipermeable membrane immersed in an aquous solution containing the small ion whose interaction is to be studied. The small ion is free to travel through the pores of the

membrane while the large protein molecules are unable to penetrate the membrane. On each side of the membrane there is a dilute solution of a strong electrolyte which prevents an unequal distribution of charges across the membrane. The existance of an unequal distribution of charges, known as the Donnan effect, would tend to shift the equilibrium established by the small ion away from its true position.

The magnitude of the interation between the protein and the small ion is obtained by a quantitative analysis of the concentration of the small ion in the solution which is separated from the protein. The analysis is carried out after equilibrium has been established. Comparison of the concentration of the small ion which is in equilibrium with the protein with the concentration of the small ion in a similar system in which the protein is absent will give directly the amount of small ion bound by the protein.

A method of calculating the maximum number of moles of a given substance which can be bound to a protein molecule has been developed by Klotz (10). This method is based on the law of mass action and assumes that the binding occurs in a stepwise fashion with the first mole bound being held the most firmly. A full derivation of the equations will not be attempted here. The complete derivation has recently been carried out by Nickel (17). The expression relating moles bound per mole of protein r with concentration of unbound ion (A) is given by

(1)

$$r = \frac{m\,(A)}{K + (A)}$$

where K is the intrinsic dissociation constant for the system and m is the maximum bound ions per protein molecule. To evaluate m and K the equation is rearranged to

$$\frac{1}{r} = \frac{K}{m}\frac{1}{(A)} + \frac{1}{m} . \qquad (2)$$

A graph of 1/r versus 1/(A) will be a straight line with the intercept on the 1/r axis equal to 1/m and the slope of the line equal to K/m.

Thermodynamic Values

Successive equilibrium constants are obtained by solving for K in equation (1) and substituting this value into

$$k_i = \frac{m\,(i - 1)}{i\,K} \qquad (3)$$

in which i is the order of binding (1,2,3,....i) and k_i is the equilibrium constant for the reversable binding of the ith mole of the protein.

The successive equilibrium constants obtained for the system are then utilized in the evaluation of thermodynamic quantities. The change in free energy (ΔF) is related to the equilibrium constant by the equation.

$$\Delta F = \Delta F^{\circ} + RT\ln Q. \qquad (4)$$

Here ΔF° is defined as the standard free energy change for the reaction in which starting reactants and final products are all

at unit activity. Here R is the molar gas constant, T is the absolute temperature, and Q is the ratio of activities of products to reactants. At equilibrium the free energy change is zero, thus

(5) $$\Delta F^{\circ} = - RT\ln k_{eq} .$$

When R is in calories per degree per mole, ΔF° has the units of calories per mole. Since the standard free energy change at a given temperature is a constant, the activity product ratio, Q, is also a constant and is defined as the thermodynamic equilibrium constant k_{eq}. The use of concentrations in place of activities in this equation probably does not lead to any appreciable error since in the low concentrations which one employed the activity coefficient is very nearly unity.

An investigation of the effect of temperature on the stability of the protein-bound ion complex is greatly facilitated by a knowledge of the entropy change ΔS. This may be obtained by a comparison of the well-known Gibbs-Helmholtz equation

(6) $$\Delta F = \Delta H + T(\partial \Delta F/\partial T)$$

with the thermodynamic expression relating free energy change and entropy

(7) $$\Delta F = \Delta H - T\Delta S$$

from which it follows

(8) $$-\partial \Delta F/\partial T = \Delta S$$

where ΔH in (7) is the change in enthalpy.

EXPERIMENTAL

The first portion of the project was devoted to the question of whether or not histamine could be bound to an animal protein in vitro, by the method of equilibrium dialysis. Since the amount of histamine bound by a protein was anticipated to be very small, a method for the quantitative determination of histamine capable of high precision and accuracy was required. Such a method was developed which seemed to fulfill this requirement.

Analytical

A stock solution of p-bromoaniline hydrochloride was prepared by dissolving 0.052 moles of p-bromoaniline in 9 ml. concentrated hydrochloric acid (sp. gv. 1.18) and diluting to 100 ml. This stock solution was found to be stable for a period of approximately one month. The diazonium salt of p-bromoaniline was prepared by reacting 1.5 ml. of the stock solution slowly with 7.5 ml. of a 5 percent sodium nitrite solution in an ice bath and diluting to 50 ml. This reagent was stable for twenty-four hours.

A characteristic azo dye was produced when the p-bromodiazonium chloride was caused to react with an alkaline solution of histamine. The color was unstable in aquous solution but was stable when extracted with n-butyl alcohol. In the quantitative procedure 2 ml. of the histamine solution with a concentration of approximately 10^{-5} moles per liter were added to 5 ml. of a 1.2 percent sodium carbonate solution contained in a 60 ml.

separatory funnel. Two ml. of the diazonium solution were added and after agitation the mixture was extracted with 10 ml. n-butyl alcohol. The color was formed in about three minutes and was stable for two hours.

Spectral absorption curves were constructed using the Beckman Model D.U. sepectrophotometer on various standard histamine solutions. These curves are shown in Plate I. The spectrophotometer was thermostated at 20°C during all measurements.

Plate I shows an absorption peak at 500 mu. A plot of optical density, log I_o/I, versus concentration is shown in Plate II. I_o is the intensity of the incident radiation and I is the intensity of the radiation after passing through the solution. Plate II shows adherance to Beer's law except at very low concetrations of histamine. It is thought that this deviation was due to monomer formation in the coupling equilibrium while more concentrated solutions were composed of polymers, possibly dimers.

Dialysis

The membrane used in this work was Visking 18/32 cellulose casing with a semipermeability that retained all protein molecules studied without hinderance to the histamine molecule.

Histamine diphosphate as purchased from Biochemicals Inc. was used throughout this work. In the determination of the purity of the histamine diphosphate it was necessary to develop a special technique in microcombustion analysis for nitrogen due to the

Explanation of Plate I

Plate I shows the effect of histamine concentration on the spectral absorption curves.

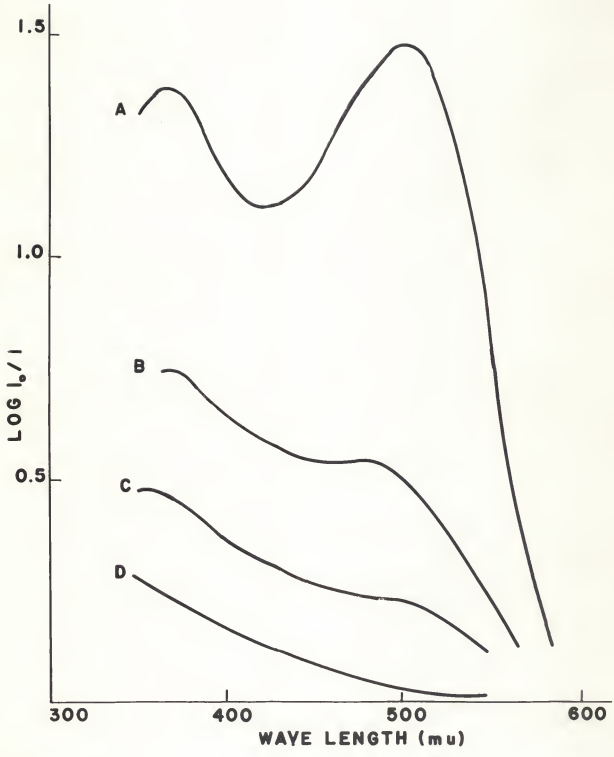
A represents 20×10^{-5} moles per liter.

B represents 10×10^{-5} moles per liter.

C represents 7×10^{-5} moles per liter.

D represents the absorption of the blank.

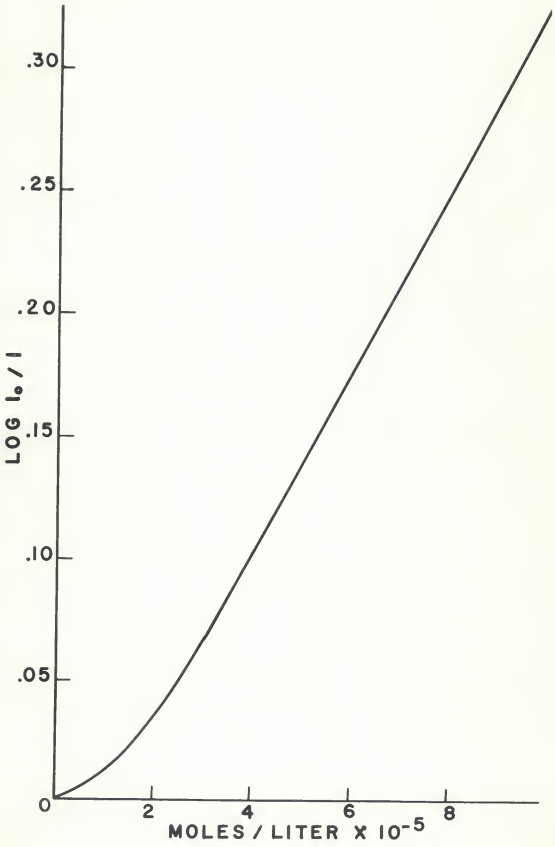
Plate I



Explanation of Plate II

Plate II shows the adherance to Beer's law for the analytical determination of histamine.

Plate II



extreme hydroscopicity of this compound. A 0.1 molar histamine solution was prepared using a vacuum dried sample of known weight. Aliquots of 0.1 ml. were then placed in preignited porcelain microcombustion boats and allowed to dry over concentrated sulfuric acid. The samples were then subjected to a microcombustion analysis for nitrogen. The results are given in Table 1. The molarity of the histamine solution was calculated by assuming that each millimole of histamine would yield 1.5 millimoles of nitrogen or 33.6 ml. nitrogen gas at standard temperature and pressure.

Table 1. Microcombustion analysis

No. :	Ml. N_2 (STP) :	Molarity
1	0.331	0.0969
2	0.279	0.0825
3	0.239	0.0706
4	0.324	0.0945
	Average =	0.0861

Using this average value the vacuum dried material was 89.6 percent histamine diphosphate.

To prevent any change in pH during the interaction between histamine and the protein which might result in a change in the binding sites available the system was buffered at a pH of 6.9. This was accomplished by preparing all protein and histamine solutions in a phosphate buffer composed of disodium hydrogen

phosphate and sodium dihydrogen phosphate with an ionic strength of 0.2.

All protein solutions were purified by out-dialysis against the same phosphate buffer solution for 36 hours. The concentration of the protein solution after out-dialysis was determined by drying a known volume at 90°C and comparing the dry weight to the dry weight of a similar volume of the buffer. Molar concentrations of the human gamma globulin solutions were calculated by using 156,000 as the molecular weight as reported by Oncley, et al (18).

Histamine Binding

Equilibrium dialysis studies involving histamine were carried out on five proteins of various animal origin. The results of these experiments have been tabulated in Table 2. The whole human blood serum was collected at Forty Riley, Kansas and represents a mixture of various types. The human gamma globulin used in this work was prepared by Cutter Laboratories, Berkeley, California. The protein concentration was approximately 0.2 percent in all experiments. The dialysis was carried out in 25 X 200 mm. test tubes containing 20 ml. of the histamine solution with 10 ml. of the protein solution contained in the semipermeable membrane. These binding studies were done in a constant temperature bath maintained at $0.0^{\circ}C \pm 0.25^{\circ}$. The dialysis cells were constantly agitated for twenty-four hours at which time the system had reached equilibrium.

Table 2. Histamine binding to various animal proteins.

Protein	Histamine concentration (Moles per liter X 10^{-5}) Blank cell	Protein cell	Moles histamine bound
Gelatin	1.56	1.31	0
	3.04	3.12	0
	6.76	6.74	0
Bovine gamma globulin	2.21	2.29	0
	5.69	5.50	0
	8.38	8.45	0
Milk whey	0.90	0.90	0
	1.76	1.76	0
	2.88	2.88	0
Human gamma globulin	2.43	2.43	0
	1.20	1.20	0
	4.28	4.27	0
Whole human blood serum	2.94	3.10	0
	3.65	3.65	0
	6.66	6.70	0

Time Study

In the course of studying the interaction of histamine with human gamma globulin, samples were withdrawn periodically from the dialysate and analyzed for histamine. The concentration at various intervals was then compared to the equilibrium concentration of histamine as determined in a blank cell. It was found that the histamine concentration in the protein systems was always lower then the initial equilibrium value. The results of some of these experiments are given in Plate III. The histamine concentration in the protein cells always returned to the equilibrium value of the blank in twenty-four hours whereas the period of maximum binding varied between six and ten hours.

Azo-Proteins

Histamine exists in solution as a cation. This was substantiated by passing a dilute solution of histamine through a cation exchange resin bed, Amberlite IR-120 (H), and observing the absence of histamine in the elute. If the process of absorption on the functional groups of an ion exchange resin is essentially the same as the reversible binding by proteins, then linking these functional groups to proteins should provide a site for histamine binding. In this manner the free energy of histamine binding to various groups on the protein could be evaluated by the method of equilibrium dialysis.

The functional groups were linked to the protein by diazo-

Explanation of Plate III

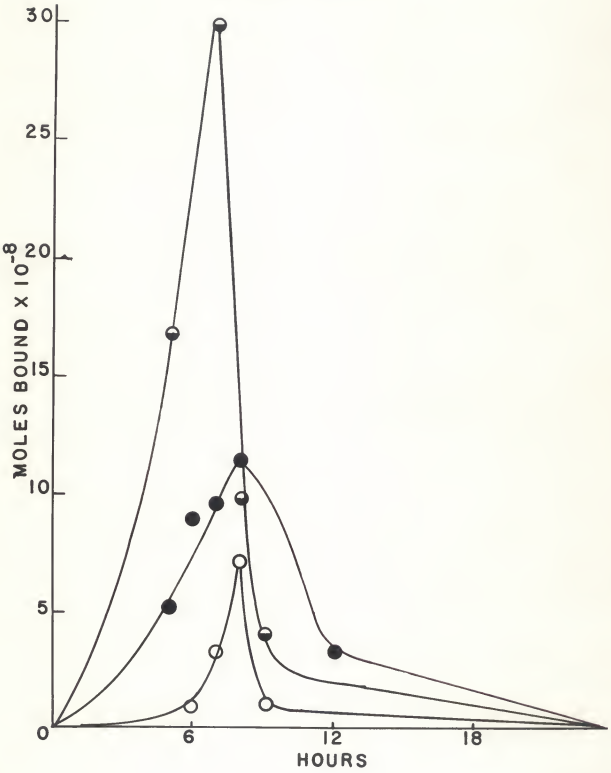
Plate III shows the effect of histamine concentration on the initial binding of histamine to human gamma globulin.

◒ 11.48 X 10^{-5} moles per liter.

○ 1.56 X 10^{-5} moles per liter.

● 3.72 X 10^{-5} moles per liter.

Plate III



tization using the method developed by Landsteiner (15). In this method the protein is coupled in alkaline solution to the diazonium salt containing the required functional group. In the resulting compound the protein is linked to the functional group by an azo phenyl linkage and the compounds are termed azo-proteins.

In this manner sulfonic, carboxylic, and arsonillic acid groups were attached to human gamma globulin. After purification by out-dialysis against phosphate buffer the azo-proteins were subjected to histamine binding by the method of equilibrium dialysis. Table 3 contains typical results of these histamine azo-protein interactions.

Reference to Table 3 shows that binding occured only when the functional group was sulfonic acid. To apply the equations developed previously for the evaluation of certain thermodynamic values histamine solutions of varying concentrations were equilibrated with the azo-protein.

Table 4 contains experimental data for the binding of histamine to the azo-protein, human gamma globulin azophenylsulfonic acid. The histamine binding as a function of temperature is shown in Plate IV.

The interaction between histamine and human gamma globulin azophenylsulfonic acid at 25° was complicated by microorganism decomposition of the histamine. All equipment and materials were sterilized by heat except the protein and the histamine diphosphate. The blank cells showed no change in histamine

concentration with time whereas the protein cells showed a gradual disappearance of histamine indicating that the micro-organisms occured only in the protein. This change in histamine concentration with time made it impossible to obtain reliable equilibrium values.

Table 3. Histamine azo-human gamma globulin interaction

Functional group : Histamine Conc. (moles X 10^{-5}) : Histamine bound (moles)
Carboxylic acid 0.79 0
2.07 0
4.42 0
Arsonillic acid 0.98 0
3.40 0
6.30 0
Sulfonic acid 1.61 0.183×10^{-6}
3.41 0.188×10^{-6}
4.75 0.213×10^{-6}

Table 4. Binding of histamine to human gamma globulin azophenylsulfonic acid.

Temperature (°C)	Histamine concentration (Moles X 10^{-5})	Moles histamine bound (per mole protein)
0.0	0.23	0.79
	0.26	0.53
	0.29	0.87
	0.35	0.88
	0.43	0.95
	0.62	1.15
	0.61	1.28
	0.86	1.60
	0.90	1.46
	1.18	1.31
	2.78	1.88
12.5	0.28	0.30
	0.43	0.36
	0.49	0.39
	0.53	0.39
	0.95	0.45
	1.66	0.36
	3.29	0.45
	4.90	0.48

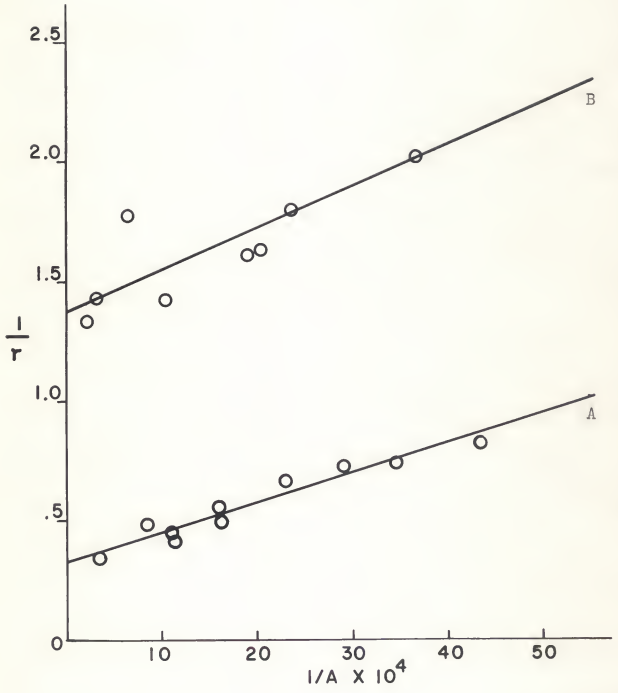
Explanation of Plate IV

Plate IV shows the effect of temperature on the binding of histamine to human gamma globulin azophenylsulfonic acid.

A represents 0.0°C.

B represents 12.5°C.

Plate IV



Binding of Methyl Orange

The absence of histamine binding to human gamma globulin as shown in Table 2 indicated that the surface forces on the protein were not negatively charged in the pH range studied. Further elucidation of these surface charges was accomplished by an equilibrium dialysis study of the methyl orange-human gamma globulin interaction since methyl orange exists in solution as an anion.

Methyl orange concentrations were obtained spectrophotometrically using the Beckman Model D.U. spectrophotometer at a wave length setting of 460 mu. This is the wave length of maximum absorption as reported by Klotz (11).

The binding of methyl orange to human gamma globulin was measured at various temperatures and the results are listed in Table 5. The effect of temperature on this binding is shown in Plate V in which 1/r is plotted against 1/(A), where r is the moles of methyl orange bound per mole of human gamma globulin and (A) is the concentration of unbound methyl orange in the system.

The binding data in Plate IV and Plate V were analyzed by the method of least squares (21). Normal equations based on equation 2 are given by

$$(9) \qquad \left[\Sigma(1/A)^2\right] K/m + \left[\Sigma(1/A)\right] 1/m = \left[\Sigma(1/r)\,(1/r)\right]$$

$$(10) \qquad \left[\Sigma(1/A)\right] K/m + N/m = \Sigma(1/r)$$

in which N is the number of observations. The values of these constants m and K are then obtained by substituting experimentally determined values into equations 9 and 10.

Table 5. The effect of temperature on the binding of methyl orange to human gamma globulin.

Temperature (°C)	Methyl orange concentration (Moles per liter X 10^{-5})	Moles bound (per mole protein)
0.0	0.337	0.084
	0.420	0.090
	0.510	0.096
	0.649	0.108
	0.815	0.111
	1.130	0.141
	1.470	0.120
	2.550	0.150
12.5	0.304	0.111
	0.527	0.126
	1.073	0.279
	1.370	0.207
	2.360	0.330
	3.930	0.660
	5.320	0.420
25.0	0.308	0.132
	0.567	0.210
	0.786	0.438
	1.073	0.330
	2.660	0.810
	3.770	1.050
	5.160	0.360

Explanation of Plate V

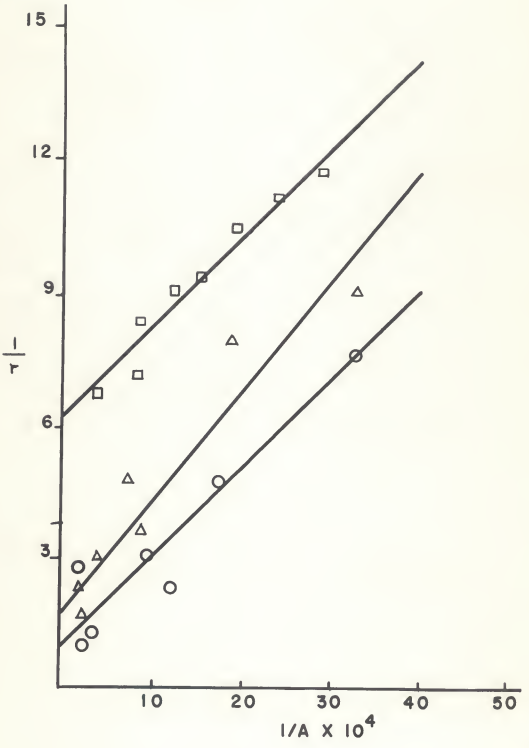
Plate V shows the effect of temperature on the binding of methyl orange to human gamma globulin.

□ represents 0.0°C.

△ represents 12.5°C.

○ represents 25°C.

Plate V



These values together with the thermodynamic values calculated from equations (5), (7), and (8) have been listed in Table 6.

Table 6. Thermodynamic values for protein binding.

Binding agent	Temp. C	m	K X 10-6	ΔF° Cal/mole	ΔS° Cal/Deg.mole	ΔH Cal/mole
Histamine	0	3.06	3.76	-1391	2	-845
	12.5	0.73	1.31	-1416		
Methyl orange	0	0.16	3.19	-1108	4	-36
	12.5	0.59	14.80	-1132		
	25	1.04	20.60	-1208		

DISCUSSION OF RESULTS

It was not possible to demonstrate any histamine binding to the five animal proteins studied by the method of equilibrium dialysis. This is in agreement with Kaplan and Davis (9) who used C-14 labeled histamine. It seems significant that the experiments utilizing specific analytical techniques for the determination of histamine showed an absence of binding whereas those experiments which have demonstrated histamine binding have utilized less specific means of analysis. The analytical procedure outlined in this work and the radioactive tracer technique mentioned above are both more specific for histamine than the biological methods which were used by Parrot, et al (19-20).

Organic anions and cations are bound to proteins by electrovalent linkages (Haurowitz, 8). A consideration of the amino acid composition of human gamma globulin indicates a preferance for anion binding rather than cation or histamine type binding. Haurowitz (8) has shown that there are 100 moles of free basic groups per 10^5 grams of human gamma globulin whereas only 67 moles of free acid groups per 10^5 grams of the same protein. Since histamine binding could only occur at the acid group sites this might explain why no histamine binding was observed.

The fact that methyl orange did bind to human gamma globulin further substantiates the fact that the surface charges on this protein are predominately positively charged and basic in nature.

Comparison of the binding capacities of methyl orange with

bovine serum albumin as reported by Klotz, et al (13) and with human gamma globulin has shown that the serum albumin is capable of binding a maximum of 23 moles per mole of protein and the gamma globulin 3 moles per mole of protein at 0°C. An explanation for this is also found in the amino acid content of each protein. Haurowitz (8) reported 148 moles of free basic groups per 10^5 grams of bovine serum albumin which is much greater than the 100 moles of free basic groups per 10^5 grams of human gamma globulin. Thus, since the basic amino acids form the sites for methyl orange binding, it is to be expected that bovine serum albumin should bind more methyl orange than human gamma globulin as this work has shown.

No adequate theory has been proposed to account for the initial binding and subsequent release of histamine by human gamma globulin which was demonstrated. The fact that histamine is initially bound and later released is substantiated by the symptoms of many allergic reactions. Food allergies, such as hives, and hay-fever seem to follow such a pattern in which the allergic reaction disappears in a matter of hours. An explanation of this phenomonen would seem to envolve a change in the structure of the protein, due to the complex formed with histamine, which results in the breaking of this complex and the release of the histamine. The protein evidently returns to its original structure in the absence of histamine since the binding capacity is restored by out-dialysis against buffer.

A possible mechanism for the binding of histamine in the

animal body is offered by the histamine binding capacity of the human gamma globulin azophenylsulfonic acid. Thus a mediating substance might be present in the animal system which when attached to the protein would provide a site for histamine binding. A small metallic ion such as ferric iron might act as a mediating agent.

The low free energy of formation of the histamine-human gamma globulin azophenylsulfonic acid complex indicates that the histamine is loosely held. Any factor which would tend to shift the equilibrium away from the complex formation would thus free the bound histamine. This might be the mechanism by which antihistamines exert their force in overcoming allergic reactions.

The positive entropy of binding might be explained by a disorientation and unfolding of the protein chain. Another explaination which has been offered by Klotz and Urquhart (12) is that the binding results in the release of water molecules held by the protein near the sites of binding. This causes an increase in the number of molecular species upon formation of the histamine-protein complex thus accounting for the positive entropy.

PROPOSED EXTENSIONS

Further attempts to bind histamine should be carried out on human gamma globulin after certain groups on the protein which repell cation binding are blocked. These groups are principally lysine and arginine. Without these basic groups exerting their repelling action the free acid groups on the protein might then

bind histamine. The effect on the binding capacity of human gamma globulin with a variation in pH before and after the basic groups are blocked should be tried.

The initial binding of histamine and subsequent release by the globulin should be extended to find the effect of certain antihistamines on this system. By introducing various concentrations of a given antihistamine to the system and observing any change in the amount of histamine bound an insight might be gained on the mechanism by which antihistamines reduce allergic reactions. Changes in the structure of the globulin during the initial complex formation might be followed by an infra-red study.

It is thought that perhaps the membrane exerts some influence on the system which opposes binding, perhaps by building up a charge from adsorbed electrolytes. Also the absence of the membrane would permit the binding studies to be carried out in a media of very low ionic strength. The large amount of electrolyte necessary to prevent the Donnan effect might block possible sites for binding. It might be possible to carry out the binding studies without the membrane by developing a chromatographic technique for the quantitative determination of histamine in the presence of the protein. In this manner the amount of unbound histamine in the system could be determined and any binding that occured would be easily discovered.

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POSSIBLE MECHANISMS OF HISTAMINE-HUMAN GAMMA GLOBULIN INTERACTIONS

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An attempt has been made to clarify the role of histamine in allergic reactions. It was found, using the method of equilibrium dialysis, that histamine would not bind to any of five different animal proteins. These included milk whey, human gamma globulin, gelatin, bovine gamma globulin, and whole human blood serum. Evidence was found, however, that histamine was bound initially to human gamma globulin and later released. This release occured about six to ten hours after initial binding. It was postulated that this temporary binding might occur in the animal body and give rise to an allergic reaction.

An analytical method for the quantitative determination of histamine was presented which eliminated some of the difficulties encountered in previous analytical techniques. The method consisted of the formation of an azo-dye by coupling histamine to a diazo salt and extracting the dye in n-butyl alcohol. The wavelength of maximum absorption was found spectrophotometrically to occur at 500 mu. At this wavelength the color formation followed Beer's law.

In order to better understand the nature of the surface charges on human gamma globulin the binding of methyl orange was carried out by the method of equilibrium dialysis. It was found that methyl orange formed a complex with the globulin. A comparison of the magnitude of this binding with that of methyl orange to bovine serum albumin showed a direct relationship with the amino acid composition of each protein. Thus bovine serum albumin which contains a greater number of basic amino acids had a

greater capacity to bind methyl orange. Evaluation of the thermodynamic values for the methyl orange-human gamma globulin complex showed that a rather loose bond existed between the two.

A site for histamine binding to human gamma globulin was provided by the diazotization and coupling of sulfanilic acid to the protein. Thermodynamic values were calculated for the binding of histamine to this modified protein. Mediation of animal proteins in the body similar to the type studied was offered as a possible mechanism for the allergic reaction.