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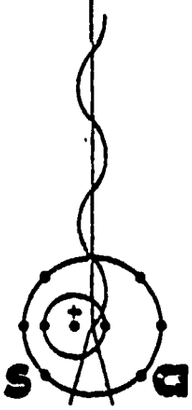

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THE MECHANISM OF HISTAMINE RELEASE FROM HUMAN BASOPHILS

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Introduction

Basophils are a type of white blood cell which comprise approximately one-half of one percent of the total blood leukocytes. Dispersed in the cytoplasm of basophils are numerous membrane enclosed granules which contain histamine and other mediators of inflammation. The mechanism of the process by which these granules are released is an easily studied example of the general phenomenon of cell activation and secretion. Recently, Dembo, Goldstein, Sobotka and Lichtenstein [1] have proposed a theory of the mechanism of histamine release. In this paper we will summarize the principal elements of this theory and explain the evidence on which these elements are based.

Cell Surface IgE

The plasma membrane of basophils has receptors which are capable of binding Immunoglobulin E (IgE) with very high affinity [2-6]. When we speak of "cell surface IgE," the presence of the Fc receptor in complex with the IgE molecule is always implied.

Each cell surface IgE molecule has two identical antigen combining sites located on the Fab fragments of the molecule. The

serum of individuals allergic to a particular antigen always contains a certain fraction of IgE, (specific IgE) which binds to this antigen. Basophils from such allergic individuals bind the specific IgE in vivo and are said to be actively sensitized. Basophils from many nonallergic individuals can be sensitized in vitro, by incubation with serum from an allergic individual; such cells are said to be "passivly sensitized."

Spontaneous dissociation of the IgE-Fc receptor complex is very slow, with a half life on the order of days. Consequently, on the time scale of the typical histamine release experiment the binding of IgE to the Fc receptor can be treated as irreversible. Further, under usual experimental conditions, the IgE in the sensitizing serum is negligibly depleted by binding to the Fc receptors. When irreversible and negligible depletion is assumed, the amount of IgE bound to human basophils after a certain time of passive sensitization is given by [6]

$$X_S(t) = \frac{C_S}{C_T} R_f[1 - \mathrm{Exp}(-r_+ C_T t)] \qquad (1)$$

In this equation, X_S is the number of IgE molecules bound per cell, C_S and C_T are the concentrations of specific and total IgE respectively during passive sensitization; r_+ is the forward rate constant for binding of IgE to the Fc receptor; R_f is the number of free Fc receptor per cell at the start of sensitization and t is the time of exposure of cells to serum.

Figure 1 shows the agreement of Eq. (1) with some data on the binding of IgE to a purified basophil preparation obtained from a

patient with basophilia [4,6]. The value of r_+ deduced from these data is shown in Table II. The values of R_f for various cell donors can be determined by measuring the amount of IgE bound to cells at saturating serum concentrations and subtracting the amount present on the cells prior to the start of sensitization. It has been found that there are large variations between different individuals in the value of R_f [4,5]. Finally, methods are available for the quantitation of C_S and C_T in various allergic sera [8], so that all of the parameters and variables in Eq. (1) can be independently determined.

The Role of Calcium

When sensitized cells are exposed to allergen in the presence of millimolar amounts of calcium, histamine is released with a time constant on the order of 1-10 minutes. The release of histamine is accompanied by the extrusion of the histamine containing granules of the basophils through a classic xocytotic process [9].

Considerable evidence indicates that the requirement for extracellular calcium, [Ca]ex, arises because a change in membrane permeability towards calcium and a consequent increase in the cytoplasmic calcium concentration are both necessary and sufficient for histamine release [1]. Furthermore, the coupling between changes in [Ca]ex and corresponding changes in the rate of histamine release is apparently very rapid. Thus, chelation of free calcium by addition of excess EDTA causes cessation of histamine release within a matter of seconds [10,11]. Conversly, readdition of calcium (but not magnesium) causes release to start again, also on a very rapid time scale.

Desensitization

As discussed above, histamine release does not occur in the absence of extracellular calcium. It has been observed however, that if calcium is added to basophils after some period of preincubation with allergen, the amount of histamine ultimately released progressively decreases to zero as the time of preincubation increases [12-14]. This progressive decrease in histamine release due to incubation with allergen in the absence of calcium is known as desensitization. As with release, desensitization has a time constant on the order of 1-10 minutes. Clearly however, unlike release, desensitization does not require the presence of extracellular calcium. It has also been found that desensitization is less sensitive to temperature than is release and that in cells of some individuals desensitization can occur at very low or so called "subthreshold" allergen concentrations, which are too low to produce detectable release [10].

Under certain circumstances, cells which have been desensitized by exposure to a particular antigen lose their ability to respond to other antigenic stimuli as well. This is known as "nonspecific desensitization." In other cases only the ability to respond to the desensitizing antigen is lost, a phenomena known as "specific desensitization" [13,14]. Neither specific nor nonspecific desensitization can be explained as a simple loss of viability of the cells, since desensitized cells retain their ability to release histamine normally as a result of non-IgE mediated stimuli such as calcium ionophores [15].

A factor found to be associated with whether desensitization will turn out to be specific or nonspecific is the number of IgE molecules per basophil. Cells with large amounts of specific IgE on their surface desensitize nonspecifically. Cells with small amounts of specific IgE, such as passivly sensitized cells, or cells from allergic donors who have a low level of specific IgE exhibit antigen specific desensitization. An additional distinction between specific and nonspecific desensitization is that specific desensitization can be reversed by incubation of the desensitized cells with allergic sera whereas nonspecific desensitization cannot be reversed by this method.

In the present discussion we shall restrict ourselves to systems where desensitization is specific. Nonetheless, it is possible to generalize the theory to explain how desensitization can go from specific to nonspecific in a continuous transition as the number of IgE antibodies per basophil increases. A particular example of how such a generalized theory can be constructed is discussed in the conclusion of reference [1].

Crosslinking

It has been found that simple binding of allergen to cell surface IgE is not sufficent to produce either release or desensitization [14,16,17]. In addition to binding the allergen must "crosslink" cell surface IgE. Crosslinking in this context means that the allergen must bind to at least two IgE molecules simultaneously and thus link them together. Formation of crosslinks between cell

surface IgE molecules is possible even if there is a very low surface density of IgE because cell surface IgE is capable of diffusing laterally in the plane of the plasma membrane [18].

The involvement of crosslinking of cell surface IgE in release and desensitization arises because normal initiation of both these processes requires that two or more Fc receptors be held in close proximity to each other. The main evidence for this is that non-physiological agents such as anti-IgE, concanavalin A or anti-Fc receptor, that are capable of brining Fc receptors into close proximity by essentially artificial means, cause histamine release [19,20,21].

These observations argue strongly that it is the purely steric event of crosslink formation and not an allosteric change in the Fc receptor which is the first signal for the initiation of histamine release and desensitization [22].

Bivalent Haptens

Bisbenzylpenicilloyl 1,6 diamino hexane or $(BPO)_2$ is an example of what we shall call a symmetric bivalent hapten.* Such molecules consist of two identical haptenic groups connected by a flexible polymethylene chain. These molecules are of interest since they are clearly the simplest chemical compounds which are capable of crosslinking cell surface IgE.

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*BPO ≡ Benzylpenicilloyl group; $(BPO)_2$ ≡ BPO-NH-$[CH_2]_6$-NH-BPO

Siraganian et al [16], have shown that basophils from rabbits immunized with penicillin antigen will release histamine when exposed to $(BPO)_2$ in vitro. The bivalent structure of $(BPO)_2$ is essential for this releasing activity since monovalent BPO haptens, such as ε-BPO formyl-L-lysine, $(BPO)_1$, did not cause histamine release. In fact, $(BPO)_1$ was found to specifically inhibit $(BPO)_2$ induced release, but did not affect release du to other antigenic stimuli.

Theory of Crosslinking of Cell Surface IgE by $(BPO)_2$

Since $(BPO)_2$ is bivalent, the only type of complexes that can be formed when it interacts with IgE are linear chains or rings as illustrated in Figure (2). This Figure also shows the additional states formed when a monovalent hapten such as $(BPO)_1$ is added to the reaction. Because of the limited variety of complexes shown in Fig. (2), it is possible to make realistic mathematical models of the equilibrium and kinetic properties of the reaction of $(BPO)_1$, $(BPO)_2$ and cell surface IgE [17,23,24]. The principal assumptions of these models are that the anti-BPO IgE on the basophils is homogeneous with regard to its ability to bind IgE and that the binding of $(BPO)_1$, $(BPO)_2$ or additional IgE to linear chains does not depend on the size of the chains.

The results of computer simulations of the time course of the crosslinking reaction indicate that unless the cyclic complexes of $(BPO)_2$ and IgE are extremely stable, thermal equilibrium is reached in a matter of seconds [17]. This means that in the absence of highly

stable cyclic complexes it should be possible to remove $(BPO)_2$ from the cell by simple washing. Indeed, washing in buffer was found to stop histamine release due to $(BPO)_2$ [17]. Similar washing had no effect on release due to antigen E. This indicates that binding of antigen E is much tighter and less readily reversibel than bindiing of $(BPO)_2$.

Computer simulations also inidicate that addition of a large excess of $(BPO)_1$ to the reaction of $(BPO)_2$ and cell surface IgE causes the breakup of crosslinks within a matter of seconds even if cyclic states of extreme stability are assumed to exist. In accord with this, addition of a large excess of $(BPO)_1$ lead to cessation of histamine release on a time scale comparable to that involved in stoppage of histamine release by addition of excess EDTA [14]. (Also see Figure (5)).

Taken together these results indicate that the binding of $(BPO)_2$ to cell surface IgE is rapidly equilibrating compared to the time scale of release and desensitization of basophils and that cyclic states are negligible. It is thus unlikely that dynamic, as opposed to equilibrium properties of crosslinking will have any effect on histamine release due to $(BPO)_2$.

If ring formation is neglected, then according to the model of crosslink formation [23], the number of crosslinked antibody molecules per cell at thermal equilibrium is given by

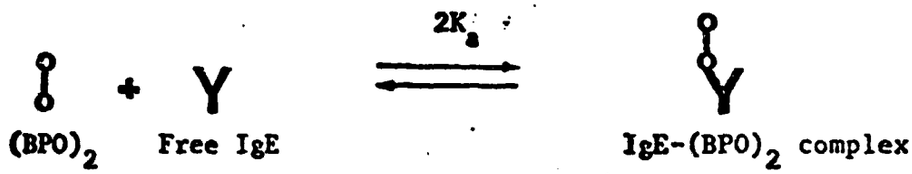
$$X_{poly}(A,B,X_S) = X_S \frac{(2S^2-2S-1+\sqrt{1+48})}{2\delta^2} \qquad (2)$$

where

$$\delta = \frac{2K_x K_a (X_s/S)A}{(1+K_a A+1/2K_b B)^2}$$

In this equation, X_S is the total number of anti-BPO IgE molecules per cell, S is the surface area of the cell and A and B are the concentrations of $(BPO)_2$ and $(BPO)_1$ respectively.

The parameters K_a (and K_b) which appear in Eq. (2) are defined in terms of the equilibrium constants for addition of a $(BPO)_2$ (or $(BPO)_1$) to previously unliganded IgE molecules,+



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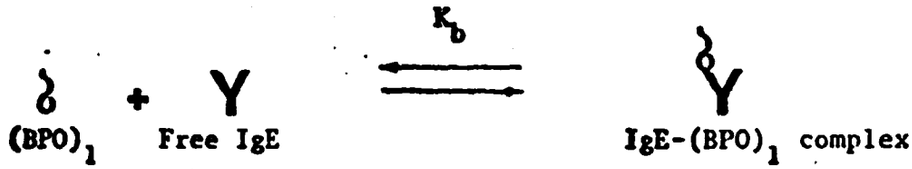

Note that we include a factor of two in the definition of K_a. This is done so that if the two BPO groups on $(BPO)_2$ each bind with the same affinity as the BPO group on $(BPO)_1$, then $K_b = K_a$.

K_x is defined as the equilibrium constant for the crosslinking reaction between free IgE and IgE-$(BPO)_2$ complex

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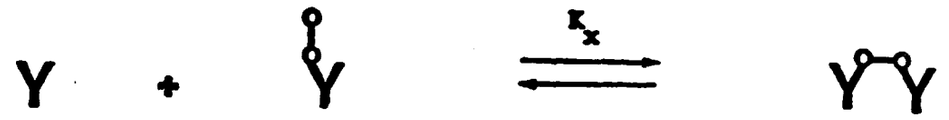
+In ref. 23 we use a different notation for the equilibrium constants. In terms of our previous notation K_a=H and K_x=V.



Free IgE IgE-$(BPO)_2$ Complex Cross-linked IgE

Analysis of Eq. (2) reveals that there are several geometric properties of a plot of X_{poly} versus logA (i.e. the cross-linking curve) which are independent of the choice of parameters. These are 1) X_{poly} approaches zero in the limit of both large and small A, i.e.

$$\lim_{A\to\infty} X_{poly} = \lim_{A\to 0} X_{poly} = 0 \qquad (3a)$$

2) X_{poly} has a single maximum which occurs at a $(BPO)_2$ concentration, A_{max}, given by

$$A_{max} = \frac{1}{K_a} + \frac{1}{2}\frac{K_b}{K_a} B \qquad (3b)$$

3) increasing the monovalent hapten concentration always decreases X_{poly}, i.e.

$$X_{poly}(A_1B_1) > X_{poly}(A_1B_2) \text{ if } B_2 > B_1 \qquad (3c)$$

4) If $A >> 1/2(K_b/K_a)B$ then X_{poly} becomes asymptotically independent of B, i.e.

$$X_{poly}(A,B) \approx X_{poly}(A,0)$$

$$\text{for } A >> 1/2(K_b/K_a)B. \qquad (3d)$$

5) The dependence of histamine release on antigen concentration is usually displayed as a plot of the percent histamine released against the $\log_{10}$ of the antigen concentration. We call such plots "histamine release curves." Analogous plots of X_{poly} versus $\log_{10}$ of antigen are called crosslinking curves. It can be shown that according to Eq. (2) the crosslinking curve is symmetric with respect to a line drawn through A_{max} parallel to the y axis, i.e.

$$X_{poly}(\log(\frac{A}{A_{max}})) = X_{poly}\ (-\log(\frac{A}{A_{max}})) \qquad (3e)$$

It has been found [25], that histamine release curves for $(BPO)_2$ in the presence or absence of $(BPO)_1$, for cells of various donors and for cells passivly sensitized with various concentrations of serum display all the properties predicted fro crosslinking curves. This observation strongly argues that Eq. (2) is a good approximate description of crosslinking and that at least for $(BPO)_2$, histamine release from passivly sensitized human basophils is a monotone increasing function of X_{poly}. In other words, histamine release increases when X_{poly} increases as a maximum when X_{poly} has a maximum and decreases when X_{poly} decreases.

If this conclusion is accepted, then Eq. (3b) provides a simple means of determining the parameters K_a and K_b. K_a was determined to be $1.2 \pm .2 \times 10^7$ ℓ/mole by simply taking the reciprocal of the concentration of $(BPO)_2$ at which maximum histamine release was osbserved, A_{max}. The ratio (K_b/K_a) was then found to be 0.38 from the shift in A_{max} produced by adding fixed amounts of $(BPO)_1$.

The value of K_x can be estimated on the basis of a statistical mechanical model of crosslink formation presented previously [25,26]. This method of estimating K_x utilizes as input parameters, the collision radius of cell surface antibody, the depth of the antibody combining site, the extended length of $(BPO)_2$ and the value of K_a. We feel that the resulting value of K_x (see Table I) is probably an overestimate although not by a large factor. The implications of the uncertainty in K_x in terms of the compensating errors introduced into the other parameters of the model have been discussed elsewhere [1].

The final unknown parameter in Eq. (2) is the surface area of the basophil, S. We have taken this quantity to be 4.7×10^{-6} cm^2 on the basis of published microscopic studies of basophils [17].

The Equation for Specific Desensitization

The theory of Dembo, Goldstein, Sobotka and Lichtenstein [1] proposes a simple mechanism to explain the occurance of specific desensitization and the dependence of specific desensitization in crosslinking. This is done by postulating the existence of an enzyme in the basophil which causes the degradation of cell surface IgE molecules if they are crosslinked to toher IgE molecules. In this way, only the IgE molecules specific for the allergen are lost during sensitization. If we assume that the enzyme operates according to Mechalis-Menten kinetics, then the rate at which specific IgE is lost due to the action of the enzyme is given by

$$\frac{d}{dt} X_S = -V_m X_{poly} / (K_m + X_{poly}) \quad . \qquad (4)$$

Since desensitization occurs in the absence of calcium, the theory also assumes that Eq. (4) is uneffected by the calcium concentration. In addition, since equilibration of IgE with $(BPO)_2$ is very rapid compared to desensitization, the crosslinking reaction will be in quasi-equilibrium. Consequently, X_{poly} will be given as a function of A, B and X_S by Eq. (2). Thus, Eq. (4) can be solved to predict the time course of loss of specific IgE from the cell surface during desensitization. As yet, this prediction has not been tested because of the difficulty of directly measuring the loss of specific cell surface IgE.

Examination of Eq. (4) reveals that as with release, desensitization is a monotone increasing function of X_{poly}. Thus Eq. (4) requires that the maximum rate of desensitization occur at the same $(BPO)_2$ concentration as the maximum of the release curve. Furthermore, as with release, the amount of desensitization after a fixed time should decrease in a symmetric manner on either side of its maximum. Both of these predictions of Eq. (4) have been previously verified [17]. (Also see Fig. ( )).

The Equation of Histamine Release

From the existence of histamine containing granules, and from the correlation between release of the granules and histamine release we conclude that histamine release occurs in quantal bursts. This is also in accord with phase microscopic observations of degranulation in which individual phase dark granules are observed to suddenly

disappear to be replaced by phase pale vesicles [9,17,28,29]. We therefore define the notion of a quantum of histamine as being a small unit of histamine, the release of which occurs in an instantaneous burst or event. Morphologically, a quantum of histane may be a single granule, some part of a granule, or a cluster of granules, as long as its constants are released more or less as a unit.

We think of the release of a quantum as being an instantaneous event, must be subject to many essentially random influences. Furthermore, since the observalbe histane release is the result of summing up a large number of such events the total rate of quantum release will be the product of the number of releasable quanta, Q_R, and the probability per unit time that a releasable quantum will be released, P_R.

$$\frac{d}{dt} Q_R = -Q_R P_R \tag{5}$$

We speak of "releasable" quanta in order to take account of the fact that a certain number of the quanta in a population of basophils may be contained in dead or damaged basophils or they may be incompetent for some other reason. We shall always assume that the total number of quanta in a population of basophils, Q_T, may be thought of as the sum of a number of releasable quanta, Q_R, and a number of nonreleasable quanta, Q_{NR}.

In light of Equation (5) the question arises as to whether or not P_R depends on Q_R. If there is some dependence of P_R on Q_R then

it means that release of different quanta are not "independent" events but that release of a given quantum can influence the probability that subsequent quanta will be released. For example, it is possible that release of a quanta could require the utilization of some chemical which is present in limited supply. In this case, release of a quantum would have a negative influence on release of subsequent quanta. Alternatively, one can conceive of situations in which quanta compete with each other for access to a limited number of release "sites." In this case release of a quantum would relieve the competition and facilitate the release of subsequent quanta.

One way in which to tell whether or not the quanta interact with each other is to examine the histamine release produced by two sequential antigenic stimuli. It is easy to show that if granule release is independent, and the signals produced by the two stimuli do not interfere with each other, then the overall release to two sequential stimuli is given by (1)

$$H_{R12} = H_{R1} + \frac{H_{R2}}{f_R}[f_R - H_{R1}] \quad . \qquad (6)$$

In this equation, H_{R1} is the release produced when the concentration of antigen two is zero; H_{R2} is the release produced when stimulus one is omitted and $f_R = \frac{Q_R(0)}{Q_T(0)}$, is the fraction of the original number of quanta which are releasable. As mentioned above, one should be aware that Eq. (6) is only applicable if the first stimulus does not interfere with the ability of the cell to receive the second stimulus. Thus the first signal should not be of a kind which nonspecifically desensitizes the cell and the second signal should

not be an antigen which crossreacts with the first.

As shown elsewhere, [1] Eq. (6) is in good agreement with data. This result supports the hypothesis that the histamine quanta of basophils are independent of each other. We note, that a particularly strong prediction of Eq. (6) is that if $H_{R1} \simeq f_R$, then $H_{R12} \simeq f_R$. In other words, if all the releasable histane is released by the first stimulus then the second stimulus does nothing. The fact that this prediction holds up even for values of f_R considerably less than one strongly indicates that a certain fraction of the histamine content of basophils is nonreleasable.

Fron the previous discussion of the role of calcium in histamine release, we see that the evidence indicates that the crosslinking signal must be transduced into a change in the calcium permeability of the cell membrane and subseuqnetly into an increased level of cytoplasmic calcium in order to induce degranulation. This clearly implies that P_R must be related to both the number of cross-linked IgE molecules on the cell surface, X_{poly}, and the extracellular calcium concentration, [Ca]ex.

In order to deduce the exact form of this relationship we first note that there is a negligible time delay between a change in X_{poly} or [Ca]ex and a corresponding change in the rate of histamine release. In other words, the transduction of a change in X_{poly} or [Ca]ex into a corresponding change in P_R must be virtually instantaneous. This means that the various intermediates in the transduction of the signal are all in rapid equilibrium with X_{poly} and [Ca]ex. Consequently, we can neglect the relaxation times associated with these intermediates

and assume that P_R can be expressed as an explicit function of X_{poly} and [Ca]ex.

Given that P_R is some explicit function of X_{poly}, the data dictates several properties which this function must possess. These are; 1) P_R must be a monotone increasing function of X_{poly} in order to have any possibility that histamine release will also be a monotone function of X_{poly}. 2) As $X_{poly} \to \infty$ there must be some point at which P_R becomes limited by factors other than crosslink availability. Thus P_R must be a saturable function of X_{poly}. 3) In order that there be a possibility of subthreshold desensitization and in order to insure that the basophil will be stable in the face of very small levels of antigen, there must be some region near the origina within which the rate of desensitization dominates the rate of release. This means that P_R must approach zero as some power of X_{poly} greater than one.

Taken together, these features imply that there is a sigmoid dependence of P_R on X_{poly}. We note, that such a relationship can be easily achieved mechanistically if a positively cooperative enzyme is involved in catalyzing some step in the transduction of a change in X_{poly} into a change in P_R.

Due to our ignorance of further details of the exact functional relationship between P_R and X_{poly} the theory empirically assumes that P_R can be parameterized by a Hill function.

$$P_R(X_{poly}) = F_{Hill} = \frac{\sigma(\frac{X_{poly}}{\Gamma})^{\alpha}}{1+(\frac{X_{poly}}{\Gamma})^{\alpha}} \qquad (7)$$

The choice of this function is motivated by its mathematical simplicity and by the fact that it satisfies the necessary criteria discussed above.

In addition to its dependence on X_{poly}, P_R must also be a direct function of [Ca]ex, the extracellular calcium concentration. Furthermore, since there is no histamine release in the absence of calcium we must require that $P_R \to 0$ as [Ca]ex $\to 0$. In light of Eq. (7), this means that $\sigma \to 0$ and/or $\Gamma \to \infty$ as [Ca]ex $\to 0$.

The quantity normally reported in histamine release experiments is the fraction of the total histamine content which is released, H_R. H_R is related to Q_R and Q_{NR} by the equation

$$H_R(t) = 1 - \frac{Q_R(t) + Q_{NR}}{Q_R(0) + Q_{NR}} = f_R - \frac{Q_R(t)}{Q_T(0)} \qquad (8)$$

By substituting from Eq. (7) and (8) into equation (5) we obtain the fundamental equation governing H_R according to the theory

$$\frac{d}{dt} H_R = \frac{(f_R - H_R)\sigma \left(\frac{X_{poly}}{\Gamma}\right)^{\alpha}}{1 + \left(\frac{X_{poly}}{\Gamma}\right)^{\alpha}} \quad . \qquad (9)$$

Quantitative comparison between theory and experiment.

Equations (1), (2), (6) and (9) in conjunction with the understanding that $P_R \to 0$ when [Ca]ex $\to 0$, are the fundamental equations of the theory [1]. This system of equations can be solved numerically for a given choice of parameter values to predict the outcome of a variety of histamine release experiments in which the antigen is $(BPO)_2$ [1].

As discussed earlier the parameters included in Equations (1) and (2) can be independently determined or deduced from very elementary properties of the histamine release curve. The remaining six parameters of the theory, V_m, K_m, σ, Γ, α and f_R here determined by means of fitting the theory to the data shown in Figures ( ) through ( ) [1]. A complete set of parameter values determined in this way are given in Table I. In figures ( ) through ( ) the solid curves show the numerical solution of the model equations for the parameter values in Table I. Thus the degree to which the theory simulates experiment can be ascertained from these Figures.

In order that the parameters of the theory be the same for all the data used for data fitting, a standard methodology was employed. Details of this methodology have appeared elsewhere [14, 25]. In addition, all data was obtained using a single source of cells and a single source of anti BPO serum. Consequently, the parameter values of Table I refer to those sources of cells and serum and may not apply to other sources.

Figure (3) shows the results of varying the concentration of serum used to sensitize the cells. Cells were incubated for ninety minutes in 1/5, 1/10 or 1/20 dilution of serum. The cells were then washed and exposed to various concentrations of $(BPO)_2$ for thirty minutes in the presence of 0.6 mM calcium. As a control, one set of cells (data indicated with +) were allwed to incubate for 15 minutes in the absence of calcium before addition of $(BPO)_2$ and calcium. The fact that such treatment has no effect on histamine release demonstrates that desensitization requires the presence of antigen and is not

an artifact due to incubation without calcium.

As can be seen, the release curves in Fig. ( ) are symmetric and all have the same maximum values. These features are clearly simulated by the model which is in good agreement with these data.

Figure (4) shows the effect of $(BPO)_2$ concentration on the extent of desensitization. Cells were sensitized in 1/5 dilution of serum for ninety minutes. Following this, cells were washed and exposed to various amounts of $(BPO)_2$ in the absence of calcium for fifteen or thirty minutes. After additional washing, cells were exposed to $10^{-7}M(BPO)_2$ for thirty minutes in the presence of 0.6 mM calcium. Also shown in this Figure for purposes of comparison is the histamine release curve for 1/5 dilution of serum from the preceding figure.

Note, that the minimum of the sensitization curves occurs at the same $(BPO)_2$ concentration as the maximum of the release curve, and that all the curves are symmetric. As discussed earlier, this suggests that cell surface IgE molecules are only degraded by the desensitizing enzyme when they are crosslinked to other IgE molecules. Also note that the minimum of the desensitization curves are very broad and flat so that near the center of the curves there is a very weak dependence of desensitization on the $(BPO)_2$ concentration. According to the model this is because X_{poly} is large near the center of the curves and the desensitizing enzyme is operating at maximum capacity.

Figure (5) shows measurments of the time course of histamine release at $10^{-7}M(BPO)_2$. Cells were sensitized exactly as in the

preceding experiment although no desensitization was carried out. Following sensitization the cells were exposed to 10^{-7}M$(BPO)_2$ in the presence of 0.6mM calcium. Release was stopped at various times by addition of excess EDTA or by addition of a large excess of $(BPO)_1$. As discussed earlier the fact that data obtained by these two methods is in agreement demonstrates that there is negligible time delay between a change in X_{poly} and the consequent change in P_R.

The various other theoretical curves in Figure ( ) show the predictions of the theory for the time course of histamine release at 10^{-8}, 3.2×10^{9} and 10^{-9}M$(BPO)_2$.

Figure (6) shows the effect of adding a constant amount of $(BPO)_1$ to the release stage of an experiment. Cells were sensitized as in the two preceding experiments. Following this the cells were washed and exposed to various amounts of $(BPO)_2$ in the presence of 0.6mM calcium and either 5×10^{-6}, 2×10^{-6}, or 3.5×10^{-7} molar $(BPO)_1$.

As can be seen, the presence of $(BPO)_1$ causes the histamine release curves to decrease and shift to the right at low $(BPO)_2$ concentrations, but has a negligible effect on release at high $(BPO)_2$ concentrations. Furthermore, the concentration of $(BPO)_2$ for maximum release, A_{max}, is shifted to the right by $(BPO)_1$ as specified by Eq. (3b). Also note that despite being shifted towards the right, the release curves remain fairly symmetric about their maxima. All these features of these data are simulated by the theory due to the fact that addition of $(BPO)_1$ has comparable effects on X_{poly}.

Conclusion

The theory of histamine release of Dembo, Goldstein, Sobotka and Lichtenstein [1] is able to explain a variety of kinetic experiments in a self consistent and quantitative way. The principal elements of the theory are:

1) The histamine content of basophils is divided into a large number of discrete and independent "quanta."

2) Release of the contents of a quanta is a stochastic event.

3) A certain fraction of the quanta in a population of basophils are nonreleasable.

4) The probability per unit time, P_R, of the release event is a sigmoid function of the number of crosslinked IgE molecules on the cell surface. In addition, P_R goes to zero when the extracellular calcium concentration goes to zero.

5) Specific desensitization is due to the action of a degradative enzyme on cell surface IgE molecules which are crosslinked to other cell surface IgE molecules. Furthermore, this enzyme is uneffected by the calcium concentration.

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Parameters of histamine release

Parameter	Symbol	Value±error	Units
number of free fc receptors per cell at the start of an experiment.	R_f	$7.3 \pm 1. \times 10^4$	$\frac{\text{molecules}}{\text{cell}}$
forward rate constant for binding of IgE to fc receptors	r_+	$3. \pm 1. \times 10^4$	$\frac{\text{liters}}{\text{mole-sec}}$
binding constant of $(BPO)_2$	K_a	$1.2 \pm 0.2 \times 10^7$	$\frac{\text{liters}}{\text{mole}}$
binding constants of $(BPO)_1$	K_b	$4.6 \pm 1.0 \times 10^6$	$\frac{\text{liters}}{\text{mole}}$
cross-linking constant	K_x	$8. \times 10^{-10} \pm$ factor of 3	$\frac{\text{cm}^2}{\text{molecule}}$
surface area of basophil	S	$4.7 \pm 1.5 \times 10^{-6}$	cm^2
maximum velocity of desensitization	V_m	1.7 ± 0.2	$\frac{\text{molecules}}{\text{cell-sec}}$
Michaelis constant of desensitization	K_m	$45. \pm 5.$	$\frac{\text{molecules}}{\text{cell}}$
maximum velocity of release	σ	$4.4 \pm 0.4 \times 10^{-3}$	$\frac{1}{\text{sec}}$
threshold of release	Γ	$360. \pm 30.$	$\frac{\text{molecules}}{\text{cell}}$
hill coefficient of release	α	1.5 ± 0.05	dimensionless
fraction of original histamine content of the cells which is releasable	f_r	0.6 ± 0.03	dimensionless

Legends to Figures

Figure 1

Binding of IgE to Fc receptors on human basophils. Reproduced from Ref. 6.

Figure 2

States fromed when a symmetric bivalent hapten, $(BPO)_2$, crosslinks cell surface IgE in the presence of a monovalent form of the hapten, $(BPO)_1$. Reporduced from Ref. 1.

Figure 3

Histamine release curves for cells sensitized with various dilutions of serum. △ and + ➡ 1/5 dilution. • ➡ 1/10 dilution and ■ ➡ 1/20 dilution. Reproduced from Ref. 1.

Figure 4

Histamine release at 10^{-7}M$(BPO)_2$ after desensitization for 15 min, ■, or 30, •, at various concentrations of $(BPO)_2$. Reproduced from Ref. 1.

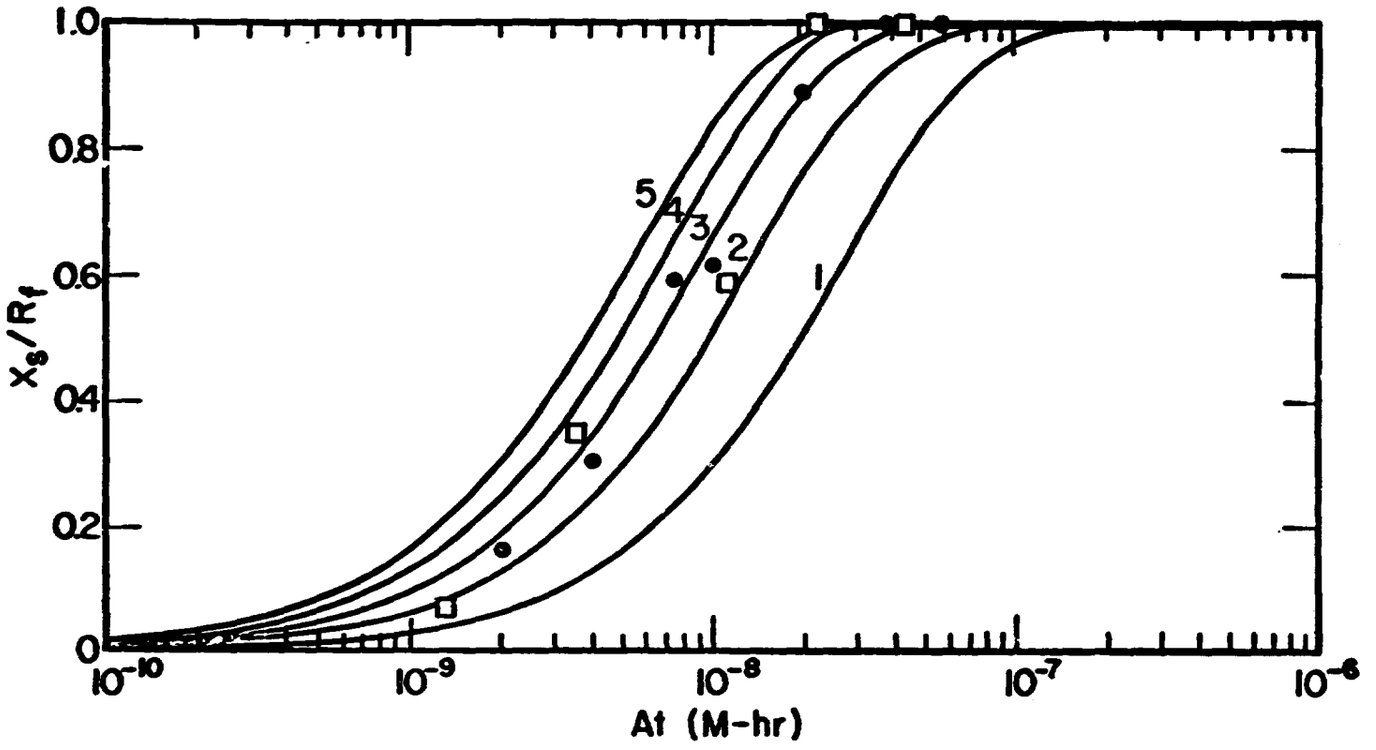
Figure 5

This course of histamine release at 10^{-7} Molar $(BPO)_2$ • ➡ release stopped by addition of 10^{-4}M $(BPO)_1$. ■ ➡ release stopeed by addition of excess EDTA. Reproduced from Ref. 1.

Figure 6

Histamine release curves in the presence of $(BPO)_1$. X ➡ $(BPO)_1$ = $5x10^{-6}$M, ♦ ➡ $(BPO)_1$ = $2x10^{-6}$M, ▽ ➡ $(BPO)_1$ = $3.5x10^{-7}$M. Also shown is the theoretical release curve for $(BPO)_1$ = 0 from Fig. 3. Reproduced from Ref. 1.

Fig 2.



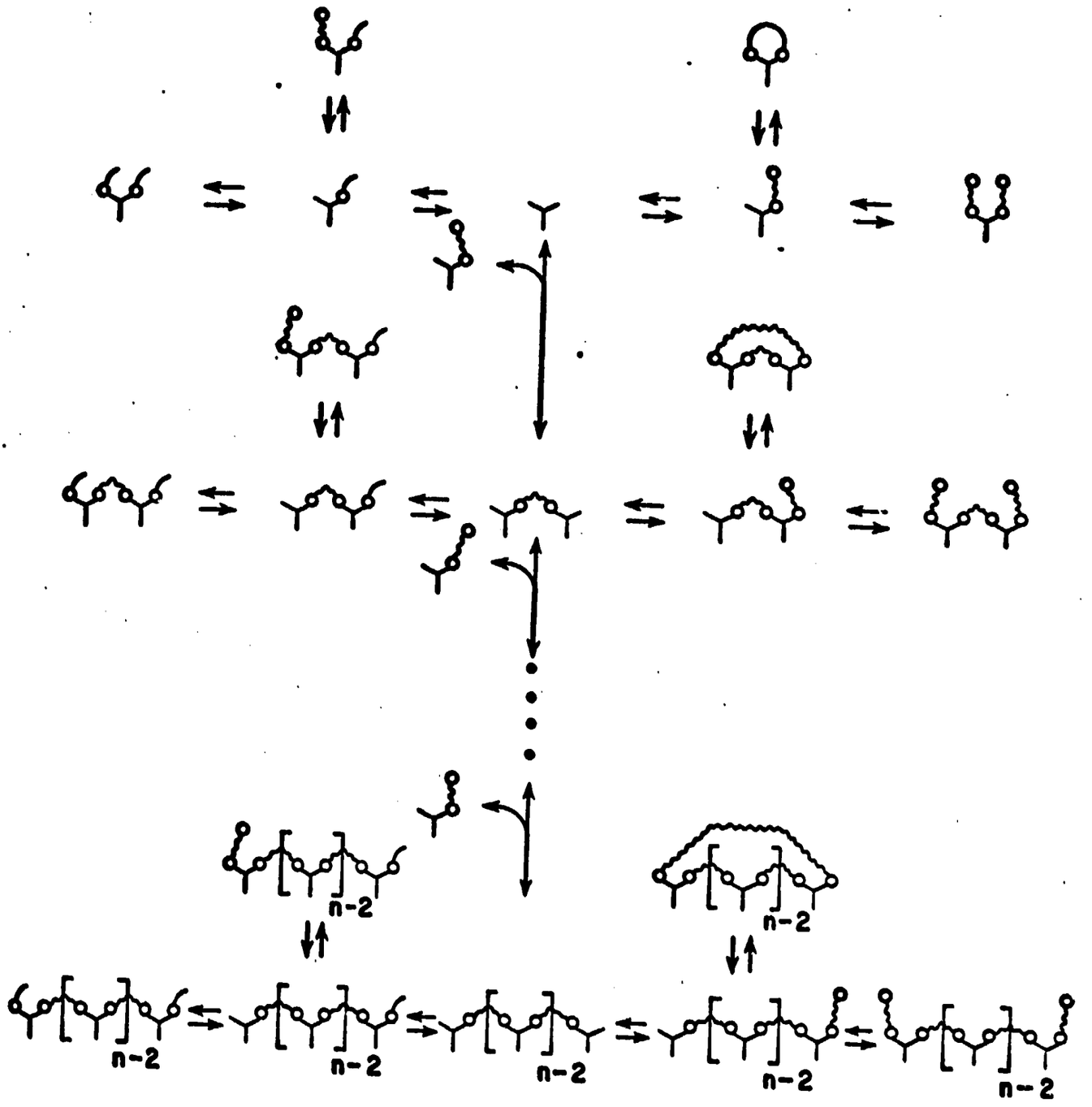


Fig2

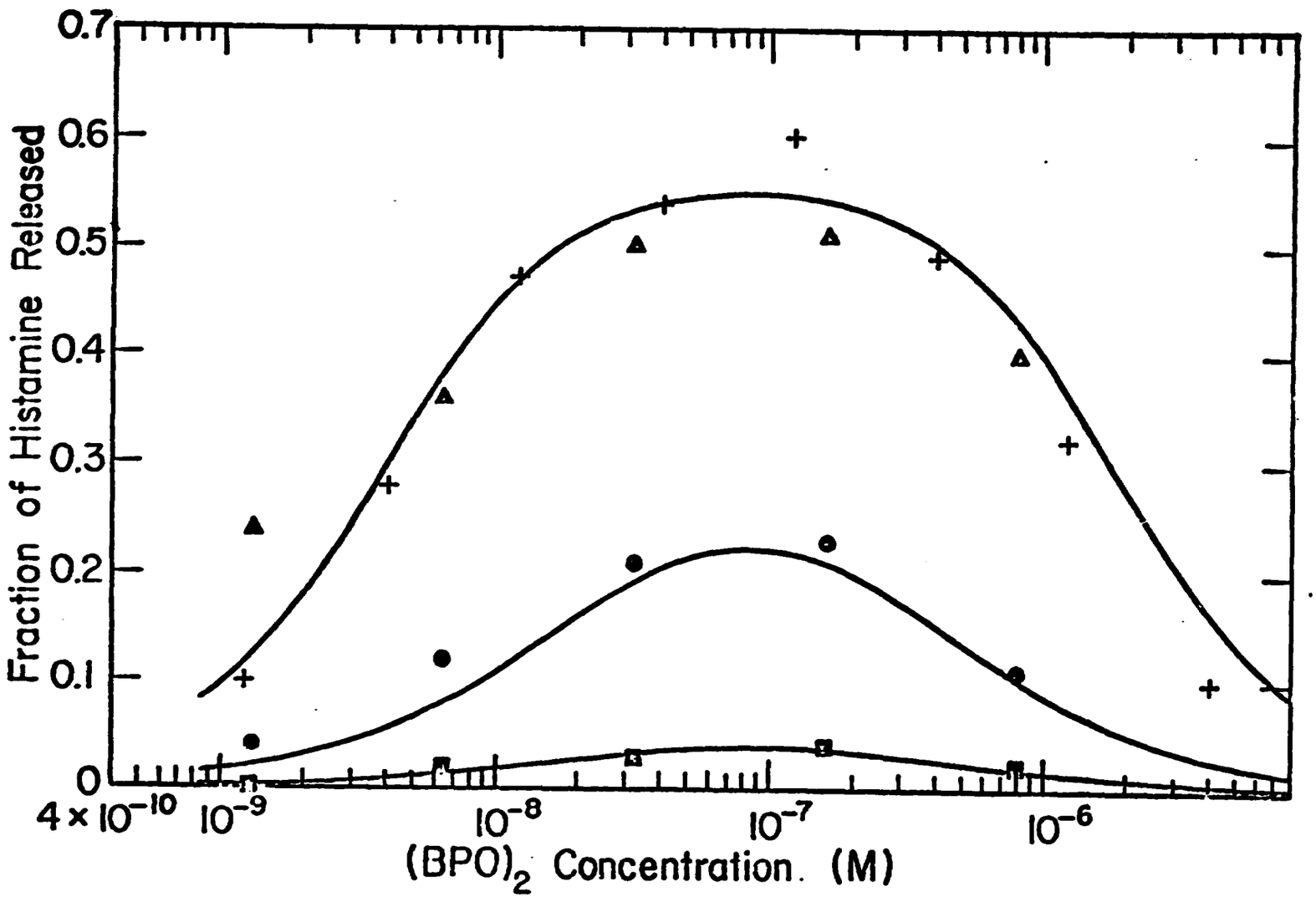

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Fraction of Histamine Released
4 × 10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
(BPO)₂ Concentration. (M)

Fig 3

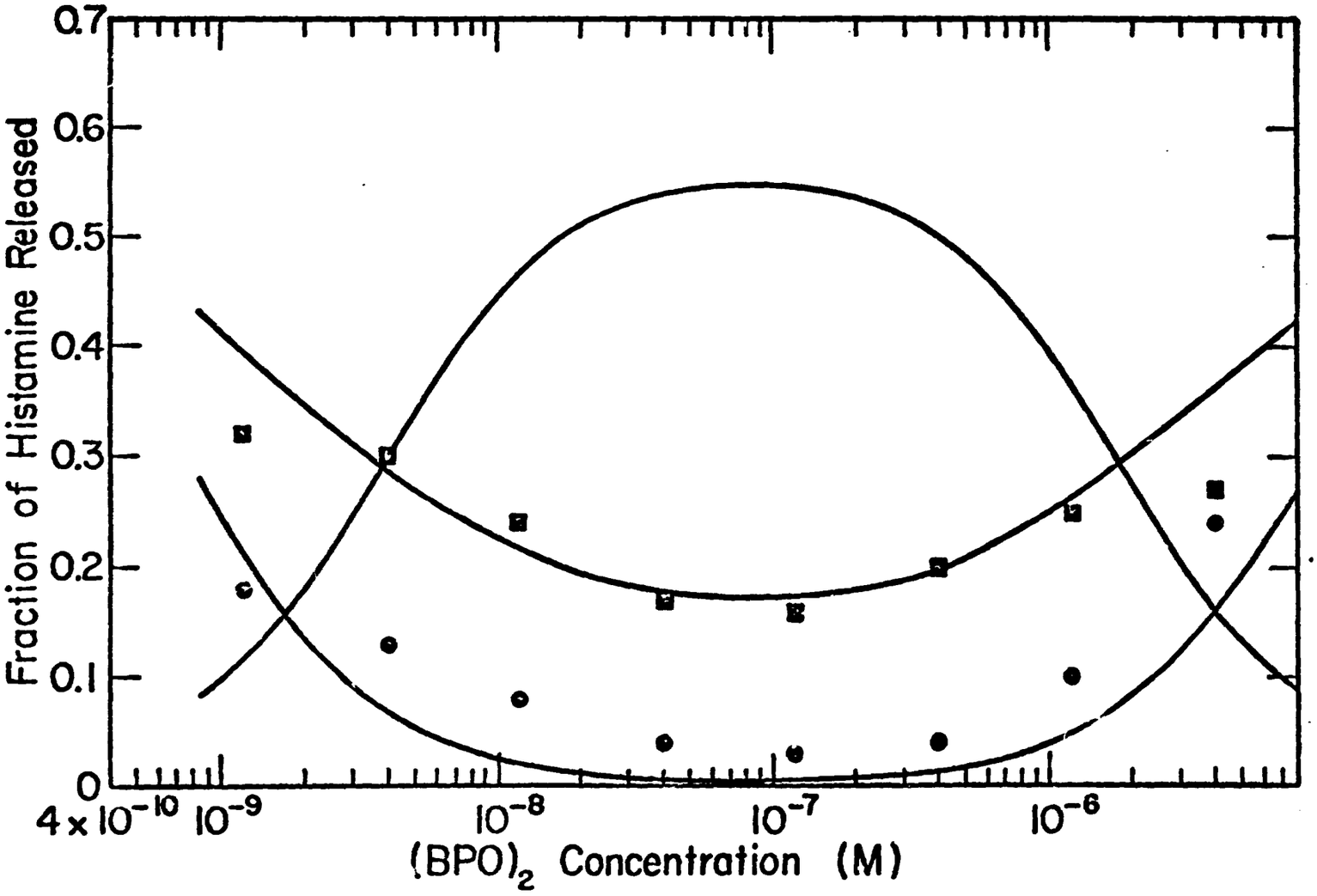

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Fraction of Histamine Released
4 x 10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
(BPO)₂ Concentration (M)

Fig 4

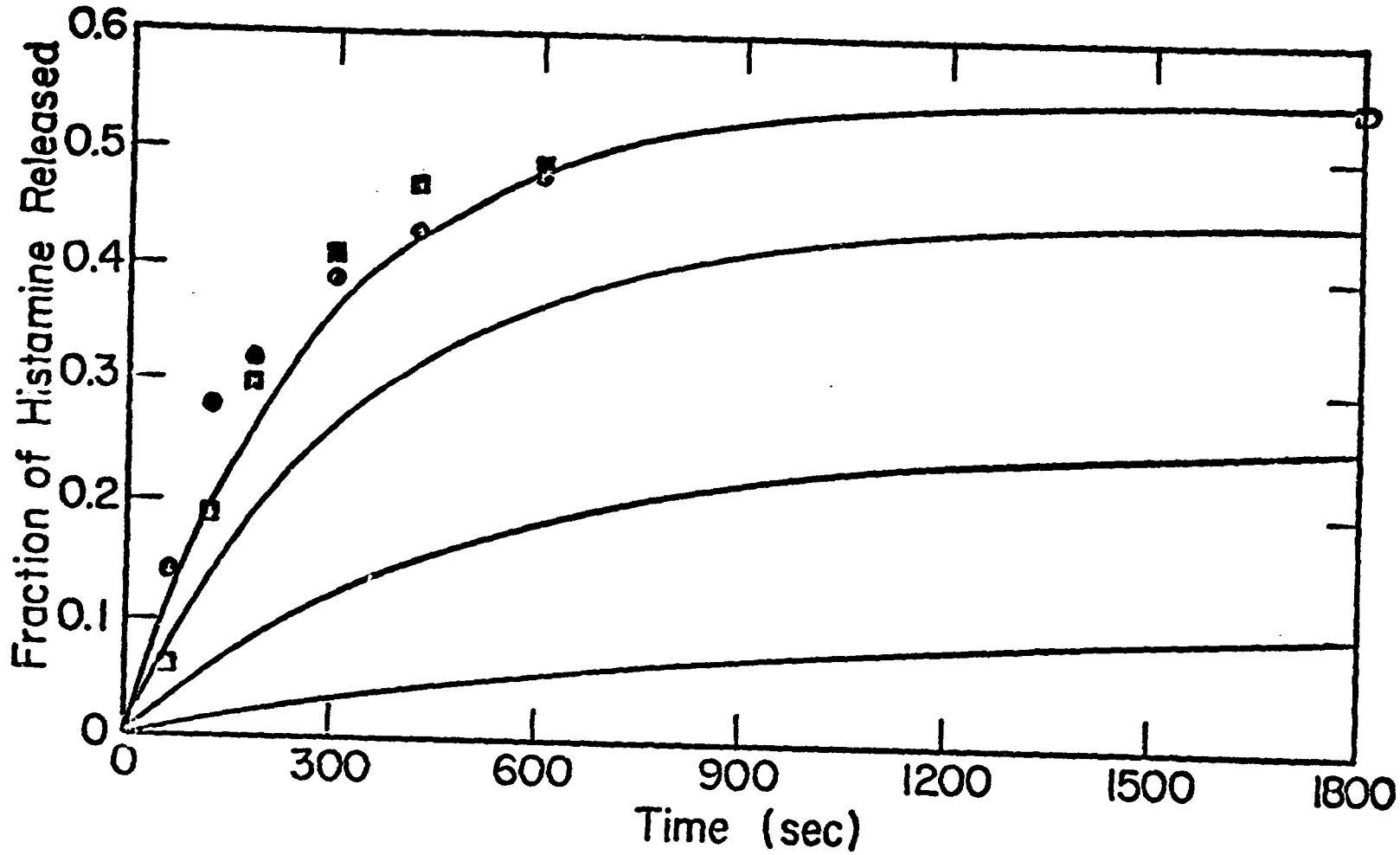

Fraction of Histamine Released
0.6
0.5
0.4
0.3
0.2
0.1
0
0
300
600
900
1200
1500
1800
Time (sec)

Fig 5

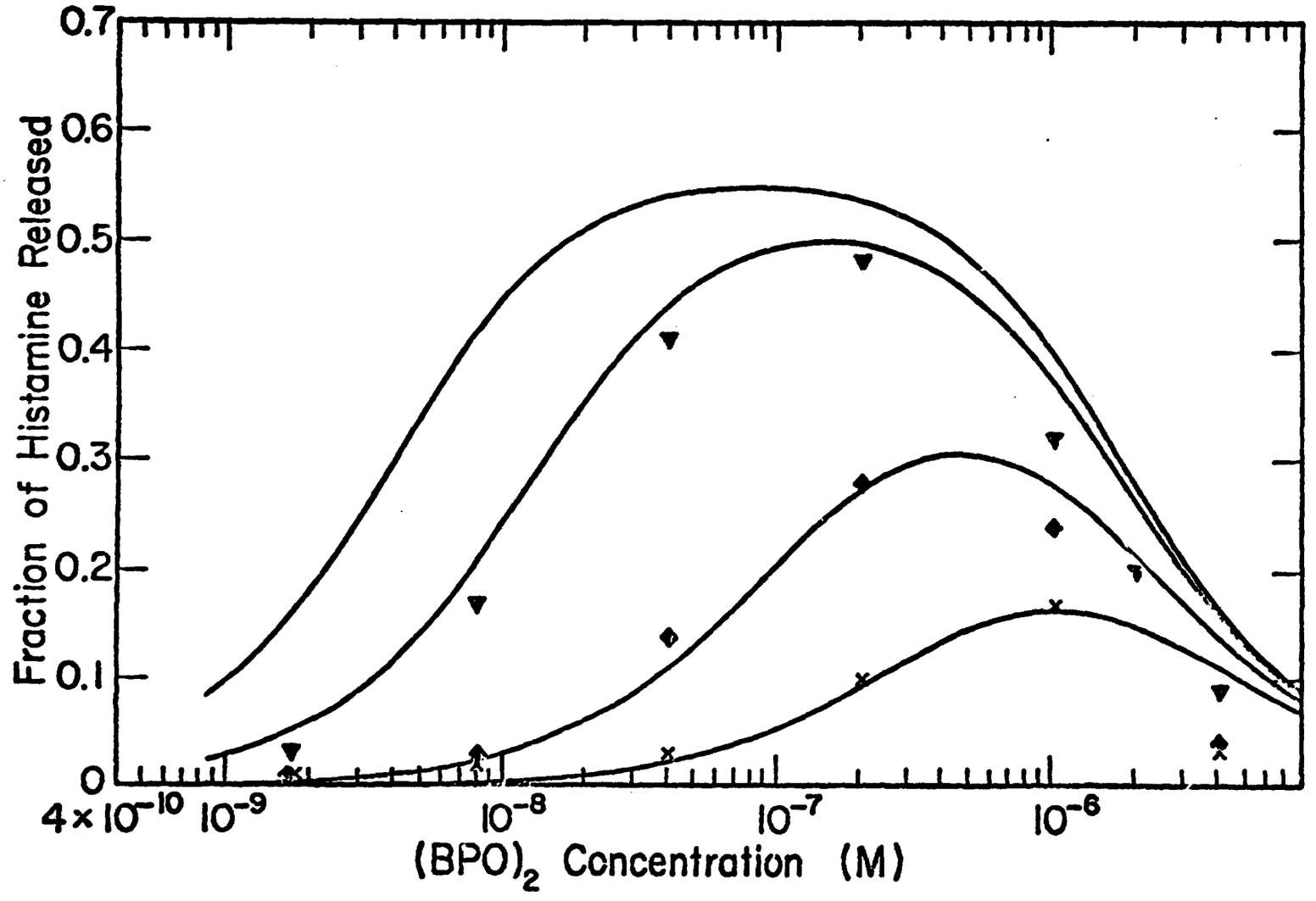

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Fraction of Histamine Released
4×10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
(BPO)₂ Concentration (M)

Fig 6