

MECHANISM OF ACTION OF CONCANVALIN A ON HUMAN BASOPHILS¹

REUBEN P. SIRAGANIAN AND PATRICIA A. SIRAGANIA

*From the Clinical Immunology Section, Laboratory of Microbiology and
Immunology, NIDR, National Institutes of Health, Bethesda, Maryland 20014*

Concanavalin A (Con A) activates human basophils to release histamine. This reaction has great similarities to that of antigen-or anti-IgE-induced release. It is characterized by a two stage reaction: the initial stage is the binding of Con A to the cell and the second stage is a secretory process of the cell. Cells desensitized by incubation with excess amounts of Con A or by prolonged incubation in the absence of cations cannot be activated to release with antigen or anti-IgE. When the native (tetravalent) Con A molecule is modified by succinic anhydride to become divalent it is much less active in releasing histamine.

There is wide variation in the response of cells from different individuals to Con A. However, a close correlation exists between the histamine release induced by Con A and anti-IgE. Therefore, in general, cells from allergic donors release more histamine with Con A than those from non-allergic individuals.

Evidence is presented to demonstrate that Con A acts by binding to carbohydrate groups on the IgE molecule located on the basophil membrane. On a molar basis, IgE is 4000-fold more active than α -methyl-D-mannoside in inhibiting the Con A-induced histamine release. Succinyl Con A blocks the ability of Con A and anti-IgE equally well and to a lesser extent the ability of antigen to activate cells to release histamine.

Concanavalin A (Con A)² has been shown to induce histamine release from the basophils or mast cells of several mammalian species (1-3). The histamine release induced by Con A from human leukocytes has great similarity to immunological activation of basophils. Both reactions require Ca^{2+} , are modulated by the level of cyclic AMP, are inhibited by the same compounds, and enhanced by heavy water (1). There is wide variation in the response of basophils of different individuals. The optimum release, however, is always obtained with 1 to 3 $\mu\text{g}/\text{ml}$ of Con A; higher concentrations are inhibitory. The Con A-induced reaction is inhibited by α -methyl-D-mannoside. This suggests that histamine release is not a result of specific IgE directed toward Con A. In contrast, antigen-induced histamine release from the basophils of atopic individuals depends on specific IgE bound to the membrane of the cell. Basophils can be activated in analogous fashion by heterologous anti-IgE.

The present experiments were undertaken to investigate further the wide variation in the response to Con A of the basophils of different individuals. There was a very close correlation in the response of cells to anti-IgE and their response to Con A. However, the release induced by Con A and allergens did not correlate as well. The interaction of Con A with the basophil was studied by the use of untreated, trypsinized, and succinic anhydride-treated Con A. Treatment of Con A with succinic anhydride or trypsin has been shown to destroy the erythrocyte-agglutinating property of Con A by splitting the molecule into its divalent subunits (4). The succinyl Con A is active in the mitogenic stimulation of mouse spleen cells. Whereas high concentrations of Con A are toxic to cells, succinyl Con A at high levels does not have this property (4).

MATERIALS AND METHODS

Solutions. Three times crystallized, lyophilized Con A was obtained from Miles Laboratories, Inc., Elkhart, Indiana. A stock 1.0 mg/ml solution was made in Tris buffer, centrifuged, and stored as 1.0-ml aliquots at -70°C . Tubes were thawed, used, and then discarded. α -Methyl-D-mannoside (α -MDM) was purchased from Phanstiehl Laboratories, Inc., Waukegan, Ill. A stock solution of ethylenediaminetetraacetic acid (EDTA) contained 0.1 M EDTA with the pH adjusted to 7.4. Lyophilized trypsin and soybean trypsin inhibitor were obtained from Sigma Chemical Co., St. Louis, Mo. Trypsin was used at a concentration of 1.0 mg/ml. Purified rabbit anti-human IgE, Fc specific, (710 μg antibody nitrogen/ml) and purified human IgE myeloma protein (PS) were kindly donated by Dr. K. Ishizaka. Rabbit anti-human IgE was also purchased from Behring Diagnostics, Somerville, N. J. The following extracts were obtained from Center Laboratories, Inc., Port Washington, N. Y.: mixed ragweed, mixed grasses, mixed trees, mixed molds, mixed feathers, mixed weeds, dog dander, cat dander, and dust. They were used over the 3 log dilutions found to be optimal for histamine release from leukocytes (5). Purified ragweed antigen E was kindly provided by Research Resources Branch, NIAID, NIH.

Modification of Con A and Rabbit anti-IgE. Con A was trypsinized using the method described by Burger and Noonan (6). The reaction was terminated by the addition of soy bean trypsin inhibitor. The method described by Gunther *et al.* was used for preparing succinyl Con A (4). Both modified Con A preparations were tested for their ability to agglutinate sheep red blood cells (SRBC). Hemagglutination of SRBC (1×10^8 cells/ml) was performed in a final volume of 200 μl in Tris ACM. The minimum concentration of untreated Con A required to agglutinate SRBC was 0.4 $\mu\text{g}/\text{ml}$. Trypsinized Con A (50 hr) at 6.0 $\mu\text{g}/\text{ml}$ and succinyl Con A at 500 $\mu\text{g}/\text{ml}$ showed no agglutination. Rabbit anti-IgE was treated with papain (7) and Fab fragments eluted from a Sephadex G-100 column. The intact mole-

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² Abbreviations used in this paper: Con A, concanavalin A; α -MDM, α -methyl-D-mannoside; EDTA, ethylenediaminetetraacetic acid; SRBC, sheep red blood cells.

cule released histamine from leukocytes at a concentration of 0.1 $\mu\text{g/ml}$ whereas the papain digested fragment was completely inactive at 12.0 $\mu\text{g/ml}$.

Buffer and Cell Preparation. The buffers and methods of cell preparation have been previously described (1). Thirty-five milliliters of venous blood were drawn into a plastic syringe, anticoagulated with EDTA, and sedimented at room temperature with clinical dextran (6% solution). The leukocytes were separated from the platelets and plasma by two washes at 4°C and finally suspended in Tris ACM (NaCl 120 mM, KCl 5 mM, Tris 25 mM pH 7.4, human serum albumin 0.03%, Ca^{2+} 1mM and Mg^{2+} 1mM). In some experiments the cells were suspended in a Ca^{2+} , Mg^{2+} -free buffer as described under results.

Reaction Mixtures. Several concentrations of Con A or anti-IgE were added in a volume of 0.25 ml to 12- × 75-mm plastic tubes (Falcon no. 2052) in an ice bath. Cells were added to all tubes in a volume of 0.25 ml and the racks transferred to a 37°C water bath. In each experiment perchloric acid at a final concentration of 3% was added to some samples (completes) to obtain the total histamine content of the cells. Blank tubes contained cells and buffer; the histamine release in these tubes was always less than 5%. At the end of 45 min the reaction was stopped by transferring the tubes to an ice bath. The tubes were then centrifuged at 1600 rpm (700 × G) for 20 min at 4°C; supernatants were poured off and assayed for histamine content. All experimental tubes were in duplicate; there was less than 2% variation between duplicate tubes.

Histamine assay. Histamine was assayed by an automated fluorometric technique recently developed by Siraganian (8). This method is an automation of the modified Shore technique for histamine determination and allows the analysis of a small sample volume with a precision of $\pm 1\%$.

Cell counts. All leukocyte and platelet preparations were examined by phase microscopy. Cell counts were performed and are recorded in the results section.

Calculations. Histamine content was determined in the experimental (e) and control (b) supernatants and the total cellular content determined after perchloric acid lysis of the cells (c). Percentage specific release was computed by using the formula: % release = $100(e-b)/(c-b)$.

Statistical analysis. The coefficient of correlation, least square line, and student *t*-test of significance were calculated by routine methods.

RESULTS

Effect of Con A on histamine release from leukocytes of 59 different individuals. Previous results (1) indicated wide variation in the response of different individuals' basophils to Con A. Figure 1 shows the wide range of histamine release from leukocytes of 59 individuals exposed to three concentrations of Con A. The donors included individuals with and without known allergies. Previous experiments have shown that 1.0 to 3.0 $\mu\text{g/ml}$ of Con A are optimal for histamine release (1). As seen in Figure 1B, almost all individuals release more histamine with 1.0 $\mu\text{g/ml}$ of Con A than with 0.3 $\mu\text{g/ml}$. There was only one individual who released no histamine to all three concentrations tested and at all these Con A concentrations there were seven individuals who released less than 5%.

Relationship between Con A, anti-IgE, and allergen-induced histamine release. Studies on cells of different individuals showed similarities in the release of histamine by Con A and anti-IgE. Figure 2 describes an experiment where one individual's cells were challenged with varying concentrations of

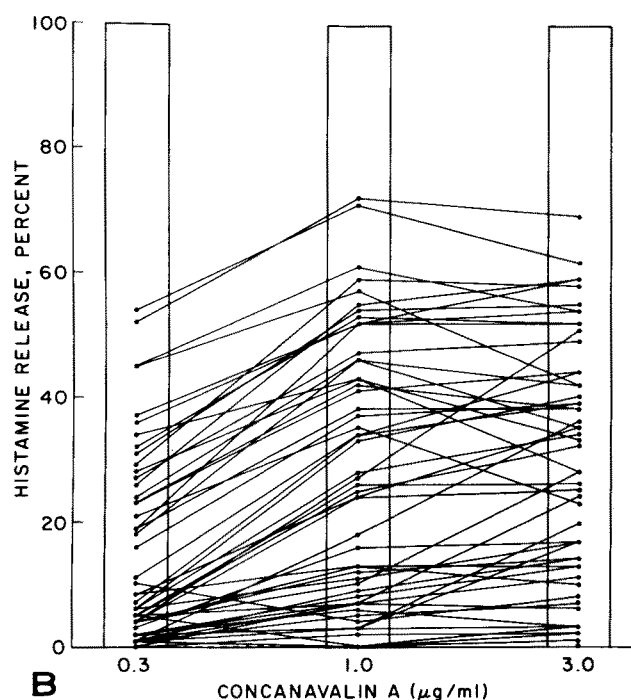
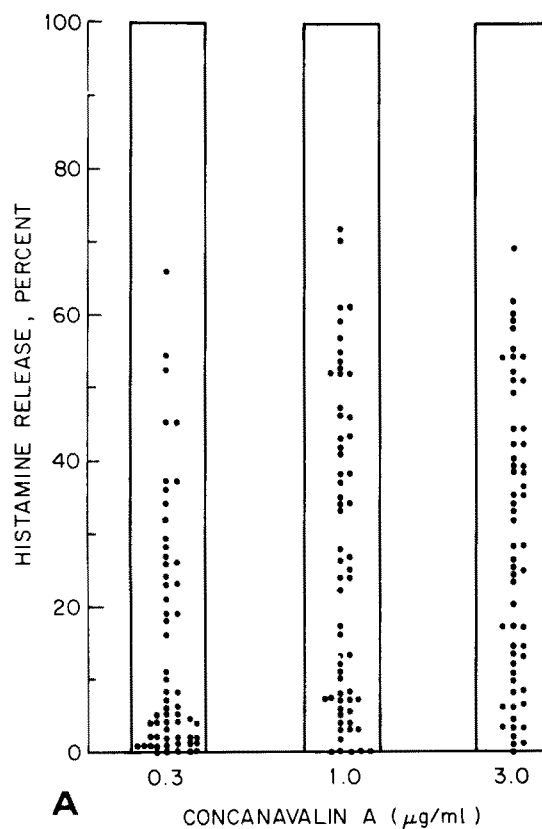
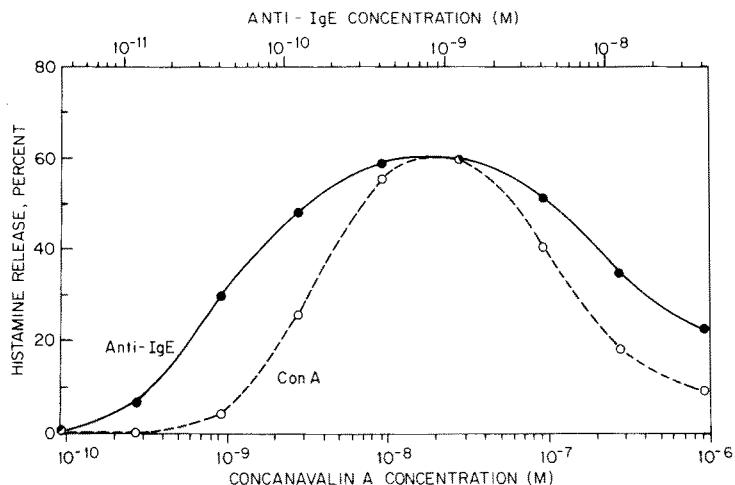


Figure 1. Histamine release with Con A from leukocytes of 59 different individuals. Washed leukocyte suspensions from 59 individuals were challenged with Con A at three different concentrations. Reaction time was 60 mins. In Figure 1A individual points are shown, whereas in Figure 1B points of each individual are joined at the three different concentrations of Con A.

Figure 2. Histamine release due to Con A and anti-IgE. Leukocytes from the same individual were activated with either Con A or anti-IgE. Reaction mixtures contained 3×10^6 leukocytes/ml.



Con A and anti-IgE. The optimal concentration of Con A was approximately 2×10^{-8} M (or $2 \mu\text{g/ml}$) and anti-IgE, 8×10^{-10} M ($0.02 \mu\text{g AbN/ml}$). A consistent finding in numerous experiments was the fact that the Con A-induced histamine release curve was not as broad as that of anti-IgE-mediated release.

The leukocytes from 38 individuals were tested with three different concentrations of Con A and four of anti-IgE (Fig. 3). This range of concentrations has been shown to give maximum histamine release. Excellent correlation was found between the maximum Con A- and the maximum anti-IgE-induced release; the correlation coefficient was $r = 0.90$, with the slope of the line = 0.83 and y-intercept of 9.9.

In contrast, a poorer correlation between Con A- and allergen-induced histamine release was observed (Fig. 4). The cells were tested with Con A (three concentrations) and nine different allergens (three concentrations each). There were eight individuals whose cells did not release histamine to any of these allergens (less than 5%). These values were not used in these correlations. The eight released maximal histamine of 1, 1, 6, 24, 34, 40, 42, and 59% with Con A. The correlation coefficient graph demonstrates that there were individuals with significant histamine release on addition of allergens with no release or minimal release to Con A ($<10\%$). The correlation coefficient was 0.76.

Inhibition of histamine release by IgE. Experiments were performed to determine if Con A-induced histamine release could be blocked by purified IgE (Fig. 5). Different concentrations of purified IgE were incubated for 40 min with either Con A or anti-IgE. Leukocytes were then added and the reaction continued for another 45 min. Both the Con A- and anti-IgE-induced histamine release could be blocked by added IgE. There was no inhibition of the anti-IgE-induced release by α -methyl-D-mannoside. However, whereas a molar ratio of IgE to anti-IgE of about 1 brought about 50% inhibition of histamine release, a ratio of 15:1 was required for the inhibition of the Con A-induced release. In studies with α -methyl-D-mannoside it was found that a molar ratio of inhibitor to Con A of more than 60,000 was required to give 50% inhibition of the histamine release.

The two stage requirement for Con A-induced histamine release from human basophils. Antigen-induced release of histamine from human basophils has been separated into at least two stages (9). In the first stage cells are rapidly activated by antigen (or anti-IgE) in the absence of Ca^{2+} in an optimal reaction time of 2 to 5 min at 37°C . Longer incubation periods re-

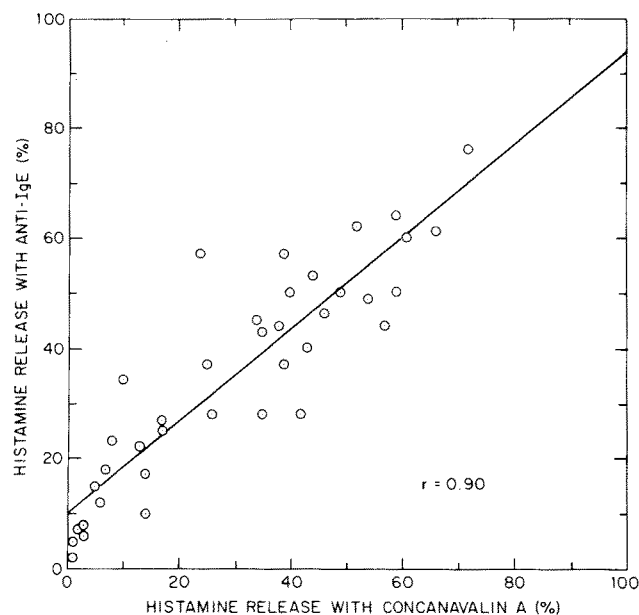


Figure 3. Correlation between Con A- and anti-IgE-induced histamine release. Washed leukocytes from 38 different donors were tested with Con A (0.3, 1.0, and $3.0 \mu\text{g/ml}$) and with anti-IgE (0.0025, 0.005, 0.01, and $0.05 \mu\text{g AbN/ml}$). The maximum histamine release with Con A and anti-IgE was used in these calculations. The linear regression line was calculated by the least square method ($y = mx + b$) where the slope of the line was $m = 0.83$ and the y-axis intercept $b = 9.9$. The linear correlation coefficient was $r = 0.90$, giving a t value of 169 which was highly significant ($p > 0.001$).

sult in a decay of the activated state. During the first stage there is no release of histamine. After washing the cells to remove excess antigen (or anti-IgE) the cells subsequently release histamine when incubated at 37°C with Ca^{2+} .

As shown in Table 1, the histamine release induced by Con A can also be separated into two stages. Leukocytes were treated with Con A at 37°C for varying periods of time and then washed (first stage). This was followed by an incubation with buffer containing Ca^{2+} (Tris ACM) (second stage). Maximal histamine release occurred with a first stage treatment of 2 to 5 min. Further prolongation of the first stage (e.g., to 40 min) significantly decreased the histamine release. Supernatants from the first stage showed no appreciable histamine release.

The leukocytes were also challenged with additional Con A during the second stage (Table I). The cells that were treated

with buffer in the first stage released between 60 and 63%; however, the cells which had been pretreated with Con A demonstrated decreased release if the first stage treatment was longer than about 9 min. Cells so treated were also incapable of releasing histamine when challenged with anti-IgE.

Further experiments were performed utilizing the optimal first stage reaction time of 4 min (Table II). Cells were incubated in the first stage with varying concentrations of either Con A or anti-IgE. When the amount of Con A in the first stage was sub-optimal there was increased histamine release on addition of more Con A or anti-IgE in the second stage. When the amount of Con A was greater than optimal there was inhibition of histamine release which could not be reversed by the further addition of Con A or anti-IgE. Similar results were obtained

when cells were treated with anti-IgE in the first stage (experiments not shown).

Effect of modification of Con A on its ability to release histamine. Trypsinization of Con A resulted in a decrease in its ability to induce histamine release from human leukocytes. As shown in Figure 6, the degree of activity loss could be correlated with the time of trypsin treatment. Untreated Con A released its maximum amount of histamine (71%) at a concentration of 1.0 $\mu\text{g}/\text{ml}$. Con A, treated with trypsin for 8 hr, released maximally (79%) at a concentration of 6.0 $\mu\text{g}/\text{ml}$. A 50-hr treatment resulted in Con A releasing 74% at a concentration of 30

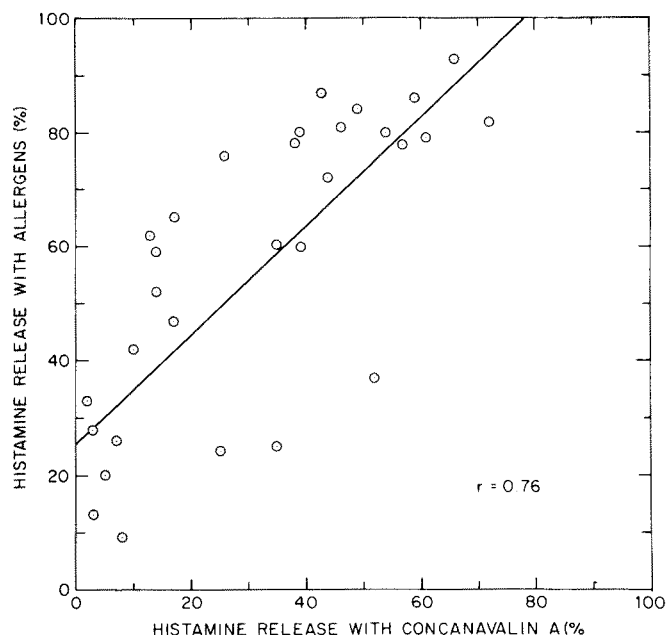


Figure 4. Correlation between Con A- and allergen-induced histamine release. Washed leukocytes from 30 different donors were tested with Con A (0.3, 1.0, 3.0 $\mu\text{g}/\text{ml}$) and with nine different allergens. The allergens were tested at three different concentrations (see *Methods* for allergens used). The maximum histamine release with Con A and allergens was used in these calculations. Individuals who did not release histamine with any of the allergens tested were excluded from the calculations. The linear regression line was calculated by the least square method ($y = mx + b$) where the slope of the line was $m = 0.96$ and the y-axis intercept $b = 25.3$. The linear correlation coefficient was $r = 0.76$, giving a t value of 55 which was highly significant ($p < 0.001$).

Figure 5. Inhibition of histamine release by IgE and α -methyl-D-mannoside (αMDM). Con A (3.0 $\mu\text{g}/\text{ml}$; 2.8×10^{-8} M) or anti-IgE (0.01 μg AbN/ml; 4.0×10^{-10} M) was pre-incubated with different concentrations of the inhibitor for 40 min at 37°C. Leukocytes were then added and the reaction was continued for 45 min. Final reaction mixture contained 2.9×10^6 leukocytes/ml. Histamine release in the absence of any inhibitor was 64% with Con A and 61% with anti-IgE.

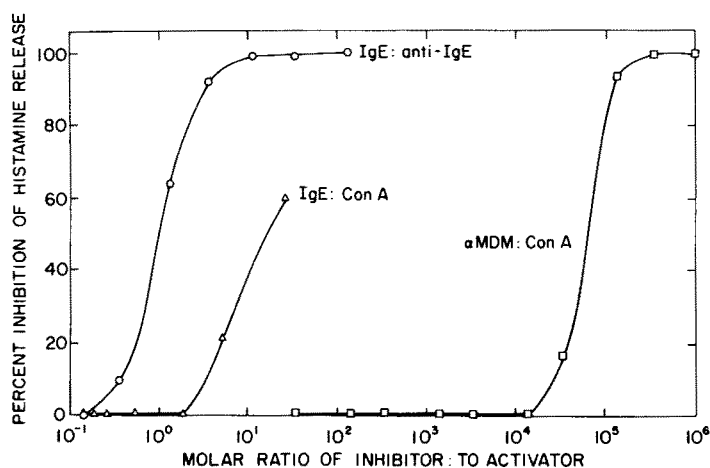


TABLE 1

The two stage requirement for Con A-induced histamine release from human basophils^a

First Stage		Second Stage		
Treatment	Time	Buffer	Con A	Anti-IgE
	min	histamine release (%)		
Buffer	10	0	60	53
	20	0	61	52
	30	0	63	51
	40	0	62	54
Con A	0.4	31	54	51
	1.3	44	60	59
	2	52	63	66
	3	51	59	60
	5	52	61	58
	7	48	54	54
	9	44	49	52
	15	31	41	41
	20	27	31	41
	30	17	26	30
	40	12	21	29

^a Leukocytes were washed twice as described in *Methods* and suspended in Tris A buffer containing 1×10^{-5} M EDTA. Cells were incubated at 37°C with either buffer alone or with Con A (2.0 $\mu\text{g}/\text{ml}$) (first stage). At indicated times (0 to 40 min), 1.0-ml samples were removed and centrifuged at $1000 \times G$ for 1 min at room temperature. The supernatant was used for histamine analysis and the cells were washed twice at 4°C with Tris A buffer. The cells were finally suspended in Tris ACM. Aliquots were added to tubes containing Tris ACM alone or with Con A (0.3 $\mu\text{g}/\text{ml}$) or anti-IgE (0.083 μg AbN/ml) (second stage). The reaction mixtures, containing 3×10^7 leukocytes/ml, were further incubated at 37°C for 45 min and the histamine release into the supernatant measured. The supernatants from the first step showed no histamine release.

TABLE II
Interaction of Con A and anti-IgE in the two stage histamine release reaction^a

Treatment in First Stage	Treatment in Second Stage						
	Buffer	Con A ($\mu\text{g/ml}$)			Anti-IgE ($\mu\text{g AbN/ml}$)		
		0.3	3.0	30.0	0.001	0.01	0.1
	histamine release (%)						
Buffer	0	38	49	16	21	52	45
Con A ($\mu\text{g/ml}$)							
0.3	1	45	58	22	26	52	51
3.0	33	44	56	36	39	53	53
30.0	12	13	15	17	11	16	32
Anti-IgE ($\mu\text{g AbN/ml}$)							
0.001	1	42	57	20	25	52	54
0.01	27	48	65	32	35	48	48
0.1	30	36	40	32	34	37	40

^a The two stage reaction was performed as described in the legend of Table I. The duration of the first stage was 4 min. Final reaction mixtures (second stage) contained 3.9×10^6 leukocytes/ml.

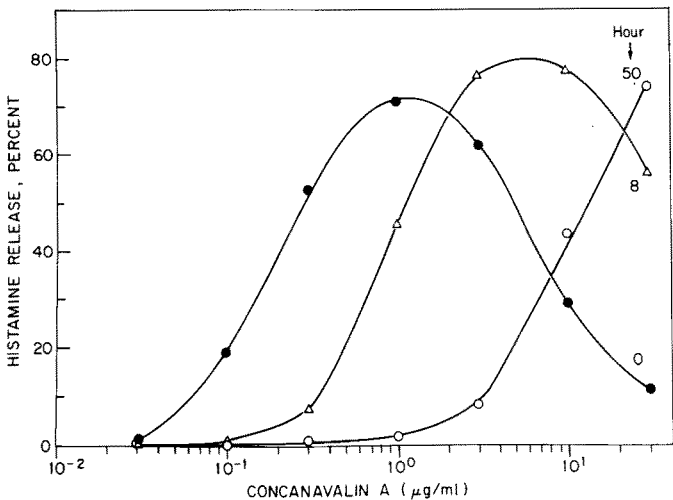


Figure 6. Histamine release by trypsinized Con A. Reaction mixtures contained 2.1×10^6 leukocytes/ml. Con A was treated with trypsin as described in *Methods*. The numbers beside each curve indicate the time period of trypsin treatment.

μg/ml. These results were parallel to the decrease in the ability of Con A to agglutinate SRBC, that is, prolonging the exposure of Con A to trypsin resulted in decreased hemagglutinating activity.

Succinylation of Con A markedly reduced its ability to induce histamine release from human basophils (Fig. 7). At a concentration of 1.0 μg/ml untreated Con A released 52% of the available histamine whereas at this same concentration succinyl Con A had no effect. In contrast, 300 μg/ml of succinyl Con A released only 36% histamine. When this succinylated Con A was tested for its ability to transform mouse lymphocytes it was active although the peak activity occurred at a higher concentration (10 μg/ml) than that produced by unmodified Con A (0.5 μg/ml).

The effect of succinyl Con A on Con A's, anti-IgE-, and anti-gen-induced histamine release. Experiments were performed to determine whether Con A reacts with IgE on the basophil membrane. Leukocytes were incubated with different concentra-

tions of succinyl Con A (30, 100, and 300 μg/ml) in the absence of added cations for 5 min. After washing, the cells were treated with different concentrations of Con A, anti-IgE or antigen. Cells from donors that released histamine to an antigen unrelated to Con A or anti-IgE were used. The results in Figure 8 demonstrate that cells pretreated with succinyl Con A during the first stage release less histamine when challenged with Con A in the presence of cations in the second stage. This effect was dependent on the concentration of succinyl Con A used. These concentrations of succinyl Con A did not cause histamine release in a two stage reaction. Parallel experiments were performed with anti-IgE (Fig. 9); the prior treatment of the leukocytes with succinyl Con A caused a marked inhibition in their response to anti-IgE. The effect of succinyl Con A on Con A- and anti-IgE-induced histamine release was identical (Figs. 8, 9) but differed from that observed when the cells were challenged with antigen in the second stage (Fig. 10). At the lowest concentration of succinyl Con A (30 μg/ml) there was some en-

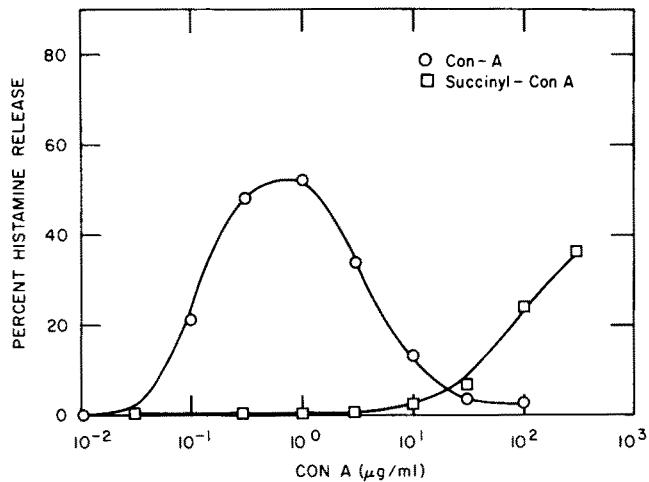


Figure 7. Histamine release with succinyl Con A. Final reaction mixtures contained 1.3×10^6 leukocytes/ml. Con A was treated with succinic anhydride as described in *Methods*.

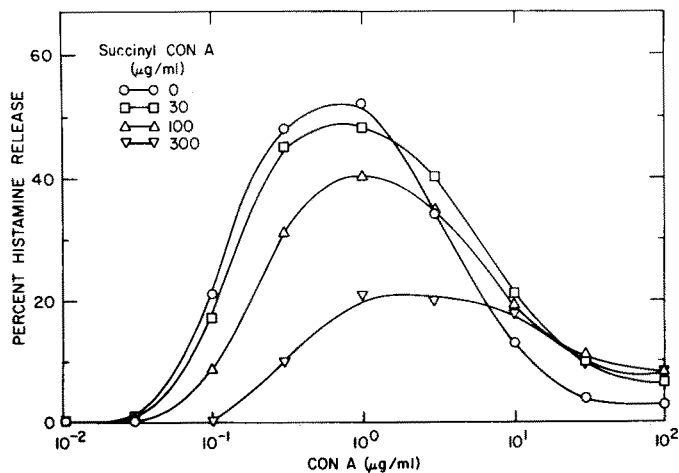


Figure 8. Inhibition of Con A induced histamine release by prior treatment of the cells with succinyl Con A. The two stage reaction was used as described in the legend of Table I. Washed leukocytes in Tris A were incubated with varying concentrations of succinyl Con A (0, 30, 100, 300 μg/ml) at 37°C for 5 min (Stage I). In the second stage these cells were incubated with varying concentrations of Con A in Tris ACM. Cells which received no Con A in the second stage released less than 3% of their histamine content (blank release).

Figure 9. Inhibition of anti-IgE induced histamine release by prior treatment of the cells with succinyl Con A. Procedure was identical to that described in legend of Figure 8 with the exception that anti-IgE instead of Con A was used in the second stage. Blanks were less than 3%.

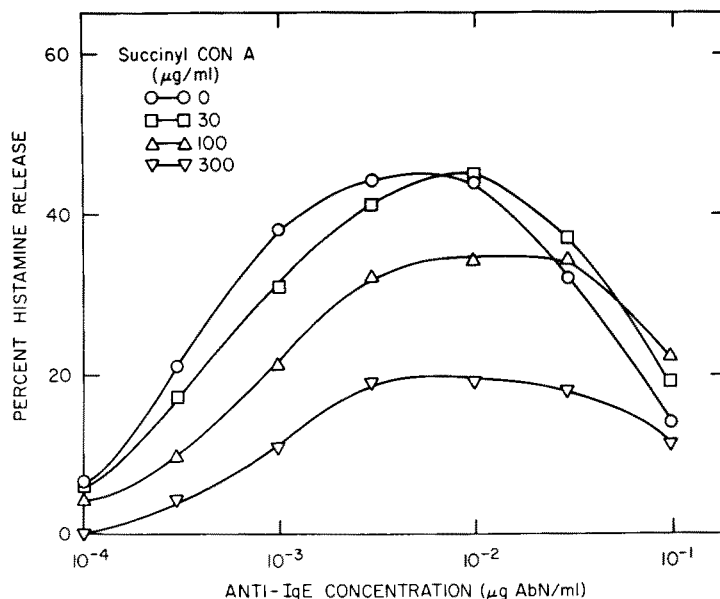
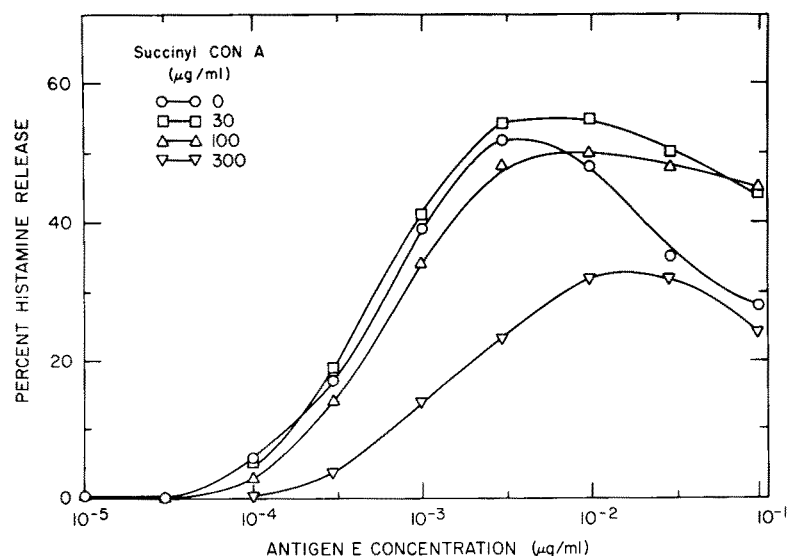


Figure 10. Inhibition of antigen-induced histamine release by prior treatment of the cells with succinyl Con A. Procedure was identical to that described in legend of Figure 8 with the exception that antigen E instead of Con A was used in the second stage. Blanks were less than 3%.



hancement of histamine release which was most marked at supra-optimal antigen concentrations, e.g., antigen E levels of 10^{-1} $\mu\text{g/ml}$ (Fig. 10). One hundred micrograms of succinyl Con A also enhanced histamine release at high antigen E concentrations although low concentrations showed slight inhibition. At higher succinyl Con A concentrations there was inhibition throughout the dose response curve.

Experiments were also performed utilizing Fab anti-IgE fragments in two-stage histamine release experiments. Leukocytes were treated with Fab anti-IgE fragments and then titrated with Con A or anti-IgE. There was no inhibition of the Con A or anti-IgE induced release by prior treatment with Fab and the release curves were nearly identical.

DISCUSSION

The wide variation in the response of different individuals' basophils to Con A is apparent from Figure 1. The results indicate a very close correlation between Con A-induced and anti-IgE-induced histamine release in response to the two stimulants (Figs. 2 and 3). Previous reports have shown that anti-IgE-induced histamine release correlates to a certain extent ($r = 0.42$ to 0.70) (10, 11) with the serum IgE levels although

that correlation was less clear than the results presented here. As a general phenomenon, anti-IgE-induced histamine release correlates to a certain extent with a person's allergic history. The histamine release curve due to Con A was consistently narrower than the anti-IgE-mediated release. This difference is probably related to the fact that Con A is tetravalent whereas anti-IgE is bivalent. The present results also show a correlation between Con A induced and allergen induced histamine release (Fig. 4); this correlation is not as good as that observed between Con A and anti-IgE induced histamine release. The results shown in Figure 4 also demonstrate that some individuals with 40 to 60% release induced by allergens release less than 20% with Con A. These cells are capable of being triggered by antigen, but Con A does not activate them. In studies of ragweed-sensitive patients the release with anti-IgE was consistently lower than the release with allergen (10, 11). The correlation between anti-IgE- and ragweed-induced release in those studies is quite similar to the correlation in the present results between allergen and Con A release. It is interesting to speculate the reason for the lower release with Con A or anti-IgE compared to the allergen-induced release. Both Con A and anti-IgE have to react with sites on the Fc portion of the

cell bound IgE. As these sites are close to the membrane they might not be as available for activating a cell as the allergen binding Fab portions of the molecule.

The release of histamine from the basophil is triggered by Con A, antigen, and anti-IgE through a mechanism which appears to be identical. The reaction in all cases requires Ca^{2+} , is temperature dependent, is inhibited by compounds which increase the level of intracellular cyclic AMP, and enhanced by heavy water (1). The release of histamine in all cases is inhibited by chlorphenesin and diethylcarbamazine. Furthermore, excess amounts of all three inducing agents inhibit histamine release.

A further similarity between Con A- and antigen-induced histamine release is the ability to divide the basophil reaction into two stages (Tables I and II). In the first stage Con A binds to the cell membrane and activates the cell in the absence of Ca^{2+} . When Ca^{2+} is added, histamine is released. The binding of Con A to sugars requires divalent cations (12). The results from these experiments suggest that the concentration of these cations required for Con A's binding to the cell membrane is less than 1×10^{-5} M. Prolonged incubation of the cells with Con A in the first stage or incubation with supra-optimal amounts of Con A results in cell inactivation, i.e., the cells will not release more histamine on addition of greater amounts of Con A or anti-IgE. Similarly, Lichtenstein showed that when cells were desensitized with one antigen they were unable to release histamine when more of this antigen or another antigen was added in the second stage (9).

High concentrations of Con A were always found to be inhibitory (e.g., Figs. 2, 6, and 7). Not only was there decreased or no histamine release, but the cells could not be triggered by a different stimulus (Table II). Yahara and Edelman (13) have found that high concentrations of Con A will restrict the mobility of lymphocyte immunoglobulin receptors. Both cap and patch formation induced by anti-immunoglobulin were inhibited. In studies with basophils, Becker *et al.* (14) found no correlation between patch-cap formation and histamine release. High concentrations of anti-IgE which were inhibitory for histamine release showed both patch and cap formation. It can be postulated that the inhibition by excess Con A is due to a membrane phenomenon with probable "freezing" of the cell surface so that other antigens cannot bring about bridging and cell activation. The two stage experiments (Table II) demonstrated that excess amounts of Con A in the first stage "froze" the cell so that it could not be activated in the second stage.

The critical event in the activation of the basophil by Con A is its binding to the cell. The activation of the basophil to release histamine requires bridging between two IgE molecules on the cell membrane. Ishizaka *et al.* (15, 16), using rabbit anti-IgE antibody, found that the monovalent Fab fragments were unable to initiate histamine release from the cell. Siraganian *et al.* (17) have demonstrated that small molecules must be divalent to activate IgE-mediated histamine release reactions. At pH 7 Con A has been shown to be a tetramer with four binding sites (4, 9). Trypsinization of Con A results in the loss of its ability to agglutinate erythrocytes and it has been postulated that trypsinized Con A is monovalent. The present findings with trypsinized Con A indicate that this treatment results in the loss of the ability of the molecule to induce histamine release (Fig. 6). Likewise, succinylation of Con A results in a loss of both its histamine-releasing activity and its ability to transform mouse lymphocytes. Previously, it has been shown that succinyl Con A is a dimer with two sugar binding sites and

that succinylation results in no loss of its ability to activate lymphocytes (4, 18). The present finding that bivalent (succinylated) Con A is much weaker than the native molecule in releasing histamine suggests either that one of the sugar-binding sites is not available to form an adequate bridge on the basophil or that the bivalent molecule is in such a steric form that one of the sites is incapable of binding to the cell. A third possibility is that the binding of tetrameric Con A has a much higher binding constant than the succinyl Con A (18, 19). Trowbridge, (18) in studies with the pea lectin, a mitogen which acts similarly to Con A, reported that succinyl- and acetyl-modified lectin showed decreased binding to spleen cells.

The fact that succinyl Con A retains its ability to bind to sugars but is unable to release histamine was utilized to study the membrane site to which Con A binds to activate the cell. Prior treatment of the cells with succinyl Con A significantly inhibited the response to anti-IgE, Con A, and antigen (Figs. 8, 9, and 10). The inhibition by succinyl Con A of Con A- and anti-IgE-induced histamine release was very similar and suggests that they are acting on sites which are very close. In contrast, the inhibition of antigen E was different (Fig. 10). At low levels of succinyl Con A there was essentially no effect on antigen-induced histamine release; at higher concentrations there was inhibition. This would suggest that the binding site for antigen is more distant than the Con A- and anti-IgE-reactive sites. It can be assumed that anti-IgE and Con A both bind to the Fc portion of the molecule whereas the antigen binds to the Fab portion. The first stage treatment of cells with succinyl Con A had an additional effect on antigen induced histamine release (Fig. 10). There was marked enhancement of release at the supra-optimal concentrations of antigen which usually cause inhibition. Similar effects were noted for the release induced by supra-optimal concentrations of Con A or anti-IgE although the effect was not as pronounced (Figs. 8, and 9). The decreased histamine release caused by supra-optimal concentrations is due to excess bridging of molecules on the basophil surface (14). Succinyl Con A by binding to IgE on the membrane inhibits bridging between molecules.

The studies with the use of Fab anti-IgE fragments demonstrate that Fab is not capable of blocking the activity of either the Con A or untreated anti-IgE. In studies with anti-DNP antibodies, Hornick and Karush *et al.* (20) have shown that the Fab has at least several logs less activity than native untreated antibody. The Fab fragment could therefore be displaced by native anti-IgE or Con A.

A crucial question is the site with which Con A interacts on the basophil membrane. Previous results indicated that the site involves a sugar, most probably a glycoprotein (1). The present experiments suggest that Con A binds to IgE on the cell membrane. There was a very close correlation between anti-IgE- and Con A-induced histamine release from the cells of different individuals. Prior incubation of Con A with IgE inhibited its ability to release histamine (Fig. 5). Purified IgE was a better inhibitor of Con A than α -methyl-D-mannoside by a factor of 4000. On a molar basis a ratio of 15:1 of IgE to Con A brought about 50% inhibition. IgE has been shown to contain 10.7 to 12.1% carbohydrate (21, 22), all of which is attached to the Fc portion of the molecule (22). Con A, by binding to this carbohydrate and bridging two IgE molecules, can activate the basophil to release histamine. However, the most direct demonstration that Con A reacts with IgE was the ability of succinyl Con A to inhibit the activity of not only Con A but also of anti-IgE.

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