MECHANISMS OF IMMUNOLOGIC INJURY OF RAT PERITONEAL MAST CELLS

I. THE EFFECT OF PHOSPHONATE INHIBITORS ON THE HOMOCYTOTROPIC ANTIBODY–MEDIATED HISTAMINE RELEASE AND THE FIRST COMPONENT OF RAT COMPLEMENT*

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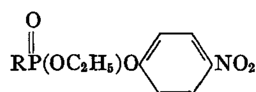
At present, the release of pharmacologically active mediators by means of an in vitro immunologic reaction requires the activity of intact, living cells. For this reason, studies of the detailed biochemical mechanism of such reactions rely heavily on the systematic and judicious use of various classes of inhibitors (1–3). Organophosphorus inhibitors such as diisopropylphosphofluoridate (DFP) and the phosphonate esters have proved particularly suitable for such studies by virtue of their ability to irreversibly and specifically inactivate the so called serine esterases (see reference 4 for references). We have recently shown that a large number of phosphonate esters of the general structure



inhibit the antigen-induced release of histamine from sensitized slices of guinea pig lung (4). These same phosphonate esters also inhibit the hemolytic activity of the first component (C′1a) of guinea pig complement (4, 5). The effectiveness of the inhibitor varies with changes in structure of the R group in a manner which is distinctly different for each reaction. On the basis of these differences, it was tentatively concluded that the antigen-antibody–activated esterase involved in histamine release from guinea pig lung was not C′1a.

The tentative nature of this conclusion stemmed mainly from the fact that histamine release in tissue slices was compared with lysis of a suspension of sensitized red cells by complement. Thus, it was possible that some of the differences noted reflected differences in the ability of the inhibitors to reach target cells in the lung tissue.

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Attention was therefore directed to the use of suspensions of rat peritoneal mast cells rather than tissue slices to study the effect of the phosphonate inhibitors on histamine release. Two quite different methods of inducing antigen-antibody–mediated histamine release from such cells have been described (6), only one of which however, is the subject of this paper. This method involves antigen-induced histamine release from well washed rat peritoneal mast cells passively sensitized in vitro (7) with the so called "mast cell lytic antibody" (8) or "anaphylactic antibody" (9) formed in the rat. This mode of histamine release is analogous to that obtained from perfused, sensitized guinea pig lung slices on the addition of antigen. Both the rat and guinea pig reactions involve the sensitization of the target cell by homologous antibody with fast electrophoretic mobility. In neither case does histamine release by antigen require serum, nor does the responsible antibody appear to mediate complement-dependent, passive immune hemolysis (9–11). In what follows, we shall term this, the "homocytotropic antibody–mediated release of histamine." The term "anaphylactic" previously used in this connection, has been abandoned since it refers to responses of the whole animal which can involve several different mechanisms.

In the present work, this form of histamine release has been studied using the phosphonate ester inhibitors. In addition, in order to assess the possible role of complement in the reaction, the activity of the phosphonate esters against the activated first component (C′1a) of rat complement was also determined.

The results obtained have permitted the following comparisons to be made: (*a*) the esterase involved in the homocytotropic antibody–mediated release of histamine from rat peritoneal mast cells has been compared with the esterase involved in the homocytotropic antibody–mediated release of histamine from mast cells of guinea pig lung slices; (*b*) the esterase involved in the homocytotropic antibody–mediated release of histamine from rat peritoneal mast cells has been compared with rat C′1a, avoiding the uncertainties introduced in our earlier studies by the use of tissue slices.

Materials and Methods

Phosphonate Inhibitors.—The *p*-nitrophenylethyl, alkyl, phenylalkyl, aminoalkyl, and the chloroalkyl-phosphonates,[1] were with few exceptions the same as used in the previous study (4). The *p*-nitrophenyl-ethyl chloropentyl phosphonate, sample 1 was the same as used previously; sample 2 was newly synthesized. The concentrations of phosphonate esters were measured in terms of the actual bound *p*-nitrophenol as determined on the day the experiment was performed.

Buffers.—The two buffers used in the study of the inhibition of C′1a, triethanolamine-buffered saline (TBS), and "low ionic strength (0.065 μ) buffer, pH 8.0" were the same as described previously (4). "Dextrose buffer" consisted of equal parts of 5% dextrose in water and TBS (12).

[1] In what follows, the *p*-nitrophenylethyl phosphonates will be named only according to the nature of the R group, the presence of the *p*-nitrophenoxy and ethoxy groups being implied.

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Tyrode's buffer with 0.1% gelatin added was used in all steps of the histamine release studies up to and including sensitization. The solution of phosphonates was prepared by adding 0.1 ml of stock inhibitor in acetone to 59.9 ml of Tyrode's gelatin solution; control Tyrode's gelatin buffer without inhibitor contained the same concentration of acetone (0.17%). This concentration of acetone was found not to interfere with antigen-antibody–induced histamine release.

Rat C'1a.—Partially purified C'1a was prepared from fresh or fresh frozen rat serum by bringing the salt concentration to 0.04 M, pH 7.5 as described in reference 12. The precipitate was dissolved in 2 times concentrated TBS, dispensed in aliquots, and stored at −20°C until required. The concentration of effective C'1a molecules was estimated as described in reference 13.

Red Cell Intermediates.—The preparation of C'2, EA, EAC'1a4, 2a was as described in reference 14. The number of EAC'1a4 sites was determined by the transfer technique of Borsos and Rapp (13). $EAC'1a^{rat}4^{gp}$ was prepared as follows: The $EAC'4^{gp}$ (1×10^9/ml) in TBS was chilled to 0°C, and rat C'1a was added in sufficient amount to give a 3-fold molecular excess of rat C'1a over EAC'4 sites. The mixture was incubated for 10 min at 0°C with occasional shaking and then centrifuged in the cold. The cells were washed 2 times with ice cold TBS. They were then washed once in dextrose buffer, and stored at 0°C in the same dextrose buffer containing 100 units of penicillin and 50 mcg of streptomycin per ml. In the preparation and subsequent use of $EAC'1a^{rat}, 4^{gp}$ no attempt was made to vary the Ca^{++} and Mg^{++} concentrations from that used with guinea pig complement despite the report that these are not optimal for rat complement (15).

Inhibition of C'1a.—The procedure for determining the activity of the various phosphonates against rat C'1a was somewhat simplified from that described previously (4). 5 ml of $EAC'1a^{rat}, 4^{gp}$ (1.50×10^8/ml, 20 to 100 effective C'1a sites per cell) in low ionic strength buffer, pH 8.0, was added to 5 ml of a 1:100 dilution of a stock solution of phosphonate in acetone, all at 25°C. By means of accurately calibrated micropipettes, 0.01 to 0.05 ml aliquots (the exact volume depending on the number of effective EAC'1a, 4 sites initially present) were removed at 3 and 10 min after mixing, and at 10-min intervals thereafter for 60 min. The samples were immediately added to 1 ml of ice cold EAC'4 (7.5×10^7 cells/ml) in TBS and stored at 1°C until sampling was complete. At the end of this time, the contents of the tubes were mixed and placed in a 30°C water bath for 15 min for the transfer reaction to occur.

A control tube containing 5 ml of EAC'1a, 4 in low ionic strength, pH 8.0 buffer with 0.05 ml of acetone added was sampled at zero time, 30 min, and 1 hr. The samples were treated exactly as described above.

A straight line relationship between the time of contact of the phosphonate with C'1a and the logarithm of the number of C'1a sites remaining was found for rat C'1a; this is the same relationship as described previously for guinea pig C'1a (4). The I_{50}, the molar concentration of phosphonate giving 50% inactivation when inhibitor and cells were allowed to stand 15 min at 25°C, and the pI_{50}, the negative logarithm of the I_{50}, were calculated as described in (4).

Antisera for Sensitization of Rat Peritoneal Cells.—Rat antisera containing antibody capable of sensitizing the rat for passive cutaneous anaphylaxis were obtained by injecting rats in each foot-pad with 0.25 ml of a mixture containing 1.0 mg dinitrophenyl–bovine gamma globulin (DNP-BGG) or five times recrystallized egg albumin (Pentex, Inc., Kankakee, Illinois) and 10^9 *Bordetella pertussis* organisms per ml.[2] The antisera were collected 7 to 15 days later and had PCA titers in the rat ranging from 1:9 to 1:81. Rat antisera against the

[2] The *Bordetella pertussis* organisms were generously supplied by Merck, Sharp, and Dohme, West Point, Pennsylvania.

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worm, *Nippostrongylus brasiliensis* (16) were generously supplied by Dr. Kurt Bloch. The details of the immunization procedure and preparation of antigen will be published elsewhere (17).

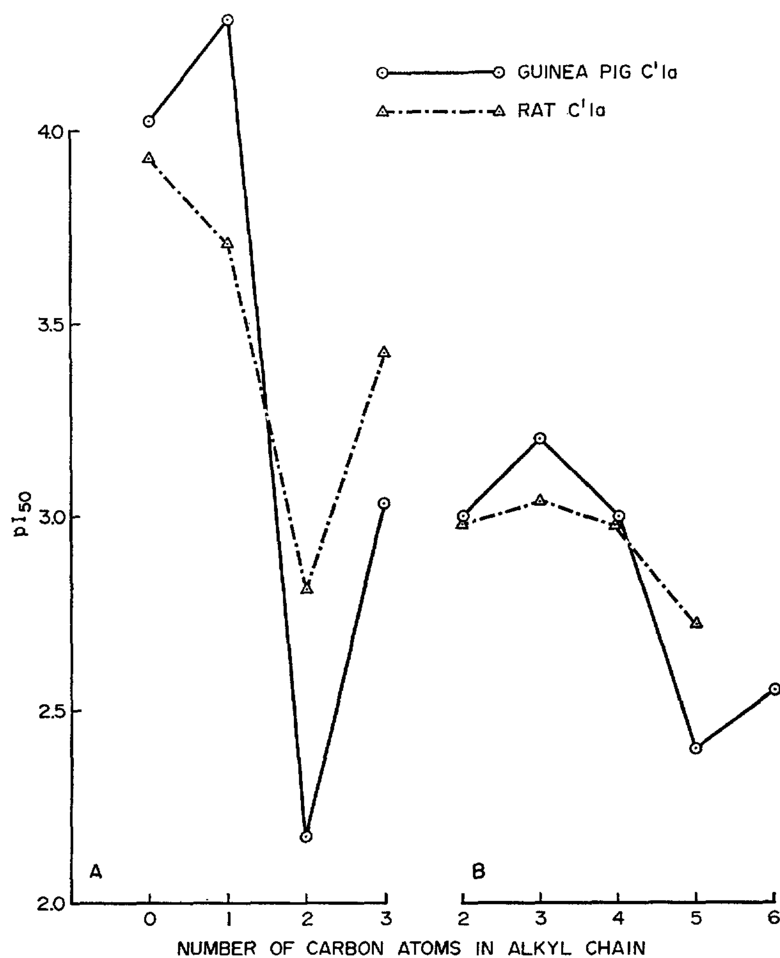

FIG. 1. The variation in effectiveness of the inhibitory activity of the *p*-nitrophenylethyl phenylalkyl phosphonate (*A*), and the *p*-nitrophenylethyl w-chloroalkyl phosphonates, (*B*) against rat C′1a and guinea pig C′1a.

Preparation of Peritoneal Cell Suspensions.—Peritoneal cell suspensions were obtained from Sprague-Dawley rats as previously described (18, 19). The cells from each rat were sedimented in plastic centrifuge tubes at 400 *g* for 2 min at 4°C, and resuspended in 2 ml Tyrode's gelatin buffer. The cell suspensions from 8 to 12 rats were then pooled, centrifuged, resuspended in 16 to 24 ml Tyrode's gelatin buffer (2 ml per rat), and washed again. The final suspension contained approximately 5% mast cells as determined by phase microscopy; evi-

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dence has been presented that the mast cells are the source of histamine liberated by the various experimental procedures (18, 19).

Homocytotropic Antibody-Mediated Histamine Release.—In a typical experiment, 24 ml of rat peritoneal cell suspension were sedimented and resuspended in a small volume of Tyrode's gelatin, usually 1.2 ml. Sensitization was achieved by incubating the cells at 37°C for 2½ hr with an equal volume of undiluted rat antiserum to DNP or egg albumin, or with a 1:3 dilution of antiserum to *N. brasiliensis*. Restriction of volume increased the rate of sensitization. It was necessary to agitate the mixture every 5 to 10 min to maintain a uniform suspension. At the end of the sensitization period the cells were diluted with 24 ml Tyrode's gelatin buffer, sedimented, and resuspended in 24 ml Tyrode's gelatin. The cells were divided into 1 ml aliquots with a volumetric pipette, sedimented, and resuspended in 2 ml Tyrode's gelatin buffer with or without phosphonate inhibitor. Following preincubation at 37°C, 0.1 ml of specific antigen was added and the reaction mixture was incubated for 7 min. The cells were

TABLE I

Inhibition of Rabbit and Guinea Pig C'1a by Pentyl Phosphonate, 5-Aminopentyl Phosphonate, and 5-Chloropentyl Phosphonate

Phosphonate	Inhibition (I_{50})	
	Rat C'1a	Guinea pig C'1a
	M	*M*
Pentyl phosphonate	1.0×10^{-3}	1.4×10^{-3}
5-aminopentyl phosphonate	3.1×10^{-6}	1.5×10^{-5}
5-chloropentyl phosphonate	1.9×10^{-3}	3.5×10^{-3}

then sedimented by centrifugation at 400 *g* for 4 min, and the supernatant fluid and cells analyzed for histamine (2). Each variable was studied using 3 cell samples, two received specific antigen, and the third which did not receive antigen served as a control for the spontaneous histamine release. Unsensitized cells from the same pool were also reacted with antigen to exclude any nonspecific histamine release by antigen alone. Duplicate samples gave values for histamine release which were within 3% of each other.

RESULTS

Effect of Phosphonates on Inhibition of C'1a.—The inhibition of rat and guinea pig C'1a by the phenylalkyl phosphonates, and by the chloroalkyl phosphonates is seen in Fig. 1, *A* and *B*, respectively. The inhibition of rat C'1a was determined for this study, that of guinea pig C'1a was taken from the previous investigation (4). The very great similarity in the patterns of the inhibition of the enzyme from the two different sources is evident in both series of phosphonates. The only real difference is that the benzyl phosphonate shows peak activity with guinea pig C'1a, whereas, with rat C'1a, the phenyl phosphonate gives maximum inhibition.

Table I shows the inhibition of rat, and guinea pig C'1a by 5-aminopentyl phosphonate, 5-chloropentyl phosphonate, and pentyl phosphonate. The 5-aminopentyl phosphonate was a much more active inhibitor of both rat and guinea

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pig C′1a than was the pentyl phosphonate, and the pentyl phosphonate was a better inhibitor than the 5-chloropentyl phosphonate.

Effect of Phosphonates on Homocytotropic Antibody–Mediated Histamine Release.—

Effect of impurities: Fig. 2 illustrates the different types of curves obtained when varying concentrations of the two available samples of 5-chloropentyl phosphonate were compared for their capacity to prevent histamine release. As in all experiments, the antigen-antibody–induced histamine release was corrected for spontaneous release. With sample 1 there was a linear relation between the per cent histamine release remaining and the molar concentration of phosphonate inhibitor; the data extrapolate back to 100 per cent antigen-induced histamine release at zero concentration of inhibitor. With sample 2 there was also a straight line relationship between the per cent histamine release remaining and the dose of inhibitor, but this line extrapolates to much greater than 100% histamine release at zero concentration of inhibitor. At the lowest concentration tested, sample 2 gave 160% enhancement.

The different dose response curves elicited by the two samples in the presence of an antigen-antibody reaction was associated with a distinct difference in the effect of these two preparations on the release of the histamine in the absence of an immune reaction. As shown in Table II, the spontaneous, or nonantigen-antibody–induced release of histamine rose greatly with increasing concentrations of sample 2; there was no such concentration dependent increase in spontaneous histamine release with sample 1.

This anomalous behavior of sample 2 can be ascribed to an impurity present in the preparation and not to the phosphonate itself since it was not observed with the other sample of the same compound. The impurity must be present in quite small amounts; gas liquid chromatography of sample 2 indicated that the compound was essentially pure.

Similar aberrant effects were found with one of the three samples of pentyl phosphonate, and in the single available samples of the 6-aminohexyl phosphonate and the 6-chlorohexyl phosphonate. Since such anomalous behavior could not be predicted from the physical properties of the phosphonates, it was necessary to set up rigid standards which a compound had to meet before it could be used for comparative purposes. Every compound was tested for and shown to meet the two following requirements before any conclusions were drawn as to its relative activity.

1. The compound had to give a linear relationship between concentration of phosphonate and per cent inhibition of immunologic histamine release which extrapolated to zero inhibition at zero concentration of inhibitor.

2. The rat peritoneal mast cells did not give an increase in histamine release when exposed to increasing concentrations of the compound in the absence of an antigen-antibody reaction.

Dose-Response Curves.—Ten compounds in all were shown to fulfill the above

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two conditions. The dose response curves given by the phenyl, benzyl, phenylethyl, and phenylpropyl phosphonates are shown in Fig. 3, *A*, and the dose-response curves for the 2-chloroethyl, 2-chloropropyl, 4-chlorobutyl, 5-chloropentyl, pentyl, and 5-aminopentyl phosphonates are given in Fig. 3, *B*.

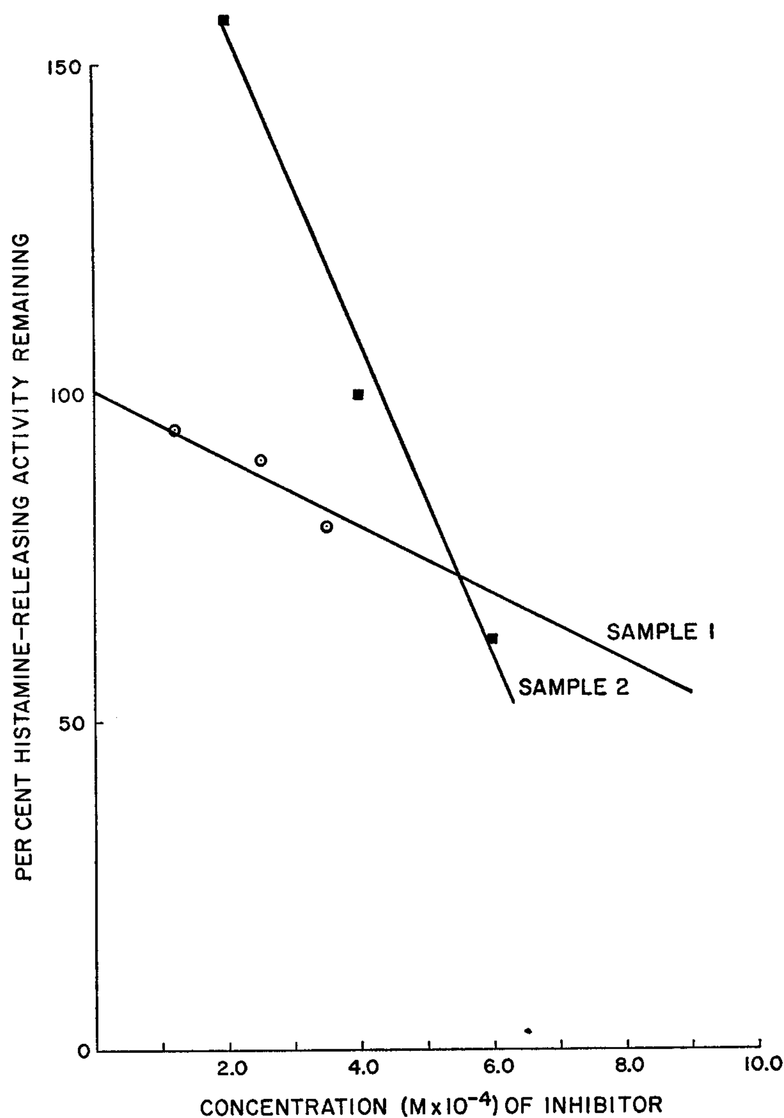

FIG. 2. The differences in the effect on antigen-induced histamine release exerted by varying concentrations of two samples of *p*-nitrophenylethyl 5-chloropentyl phosphonate. Antigen-induced histamine release in buffer without inhibitor was assigned a value of 100 per cent. Mast cells sensitized with rat antiovalbumin 2.

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In the same experiments in which the dose-response curves were obtained, the behavior of the controls with added phosphonate but without added antigen allowed us to ascertain that these same 10 compounds also fulfilled the second condition.

Demonstration that the Phosphonates Do Not Act Irreversibly on the Cells.—Mast cells were sensitized with rat antibody to the worm, *N. brasiliensis*. In one series of tubes the cells were resuspended in either 2 ml of 2×10^{-4} M phenylpropyl phosphonate, 8.0×10^{-4} M benzyl phosphonate, 6.0×10^{-4} M 4-chlorobutyl phosphonate in Tyrode's gelatin or in a control of 0.17% acetone in Tyrode's gelatin. After 5 min at 37°C, the cells were sedimented by centrifugation, washed once, resuspended in 2 ml Tyrode's gelatin with 0.17% acetone and challenged with the *N. brasiliensis* antigen. In a second series of tubes, sensitized mast cells were exposed to the same concentrations of the same inhibitors. Antigen was added while the cells were still in the presence of the inhibitors. The amount of histamine released under both these circumstances was determined as usual.

TABLE II

Effect of Two Different Samples of 5-Chloropentyl Phosphonate on the Spontaneous Release of Histamine from Rat Peritoneal Mast Cells

Sample 1		Sample 2	
Concentration	Histamine release	Concentration	Histamine release
$\times 10^4$ M	mμg/ml	$\times 10^4$ M	mμg/ml
0	20	0	20
1.3	50	2.0	80
2.5	50	4.0	160
3.5	60	6.0	250

The results are seen in Table III. Incubation of sensitized mast cells with inhibitor before, but not during the addition of antigen, had no effect on the resultant antigen-induced release of histamine. However, these same inhibitors gave 55 to 85% inhibition when present during the addition of antigen to the sensitized mast cells. In another experiment, the same three inhibitors at the same concentration gave similar results with mast cells sensitized to egg albumin.

In still another experiment, cells sensitized to DNP bovine γ-globulin were incubated with 3.0×10^{-4} M 5-aminopentyl phosphonate; after removal of the inhibitor by washing, the cells gave the expected antigen-induced release of histamine. However, 79% inhibition was obtained when DNP–bovine serum albumin was added in the presence of the aminopentyl phosphonate.

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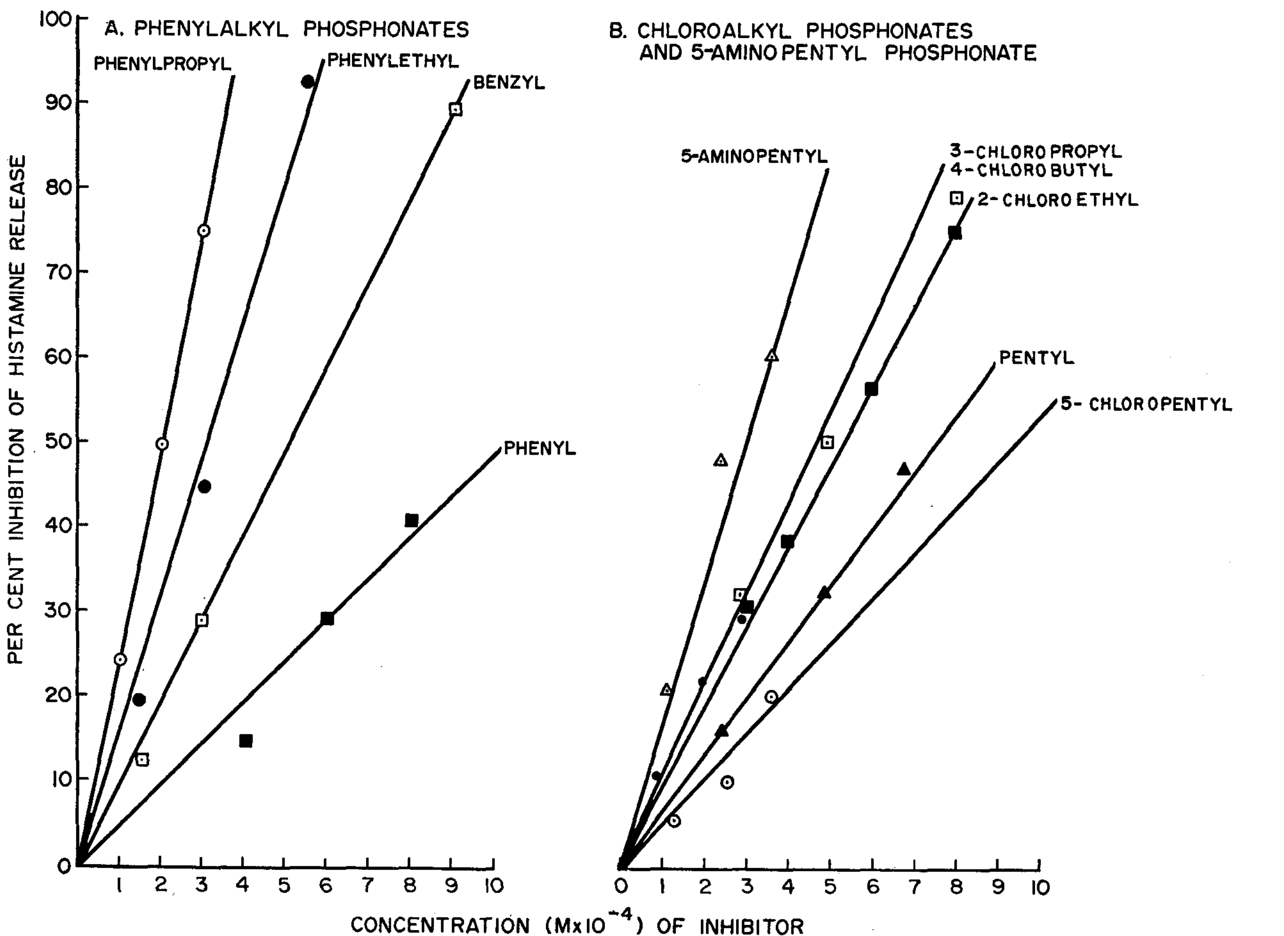

FIG. 3. The effect of the concentration of the phenyl, benzyl, phenylethyl, and phenylpropyl phosphonates (*A*), and the 2-chloroethyl, 3-chloropropyl, 4-chloropentyl, pentyl, and 5-aminopentyl phosphonates (*B*), on their ability to inhibit homocytotropic antibody–mediated histamine release from rat peritoneal mast cells. The experiments with the benzyl and phenylethyl phosphonates were performed with mast cells sensitized with rat anti-*N. brasiliensis*; those with the phenyl, phenylpropyl, 4-chlorobutyl (●) 5-chloropentyl, and 5-aminopentyl phosphonates were performed with cells sensitized with rat antiovalbumin 2, and rat anti-DNP bovine γ-globulin was used to sensitize mast cells in the experiments using 2-chloroethyl and 3-chloropropyl phosphonate (⊡).

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TABLE III

Effect of Phosphonates on the Sensitized Mast Cells in the Presence and Absence of Antigen

Concentration	Phosphonate	Histamine release as per cent of control*	
		Phosphonates present before but not during addition of antigen	Phosphonate present during addition of antigen
$\times 10^4$ M			
0		100	100
2.0	Phenylpropyl	81	45
8.0	Benzyl	106	15
6.0	4-chlorobutyl	100	29

* Control refers to antigen-induced release of histamine from replicate cells never exposed to phosphonate.

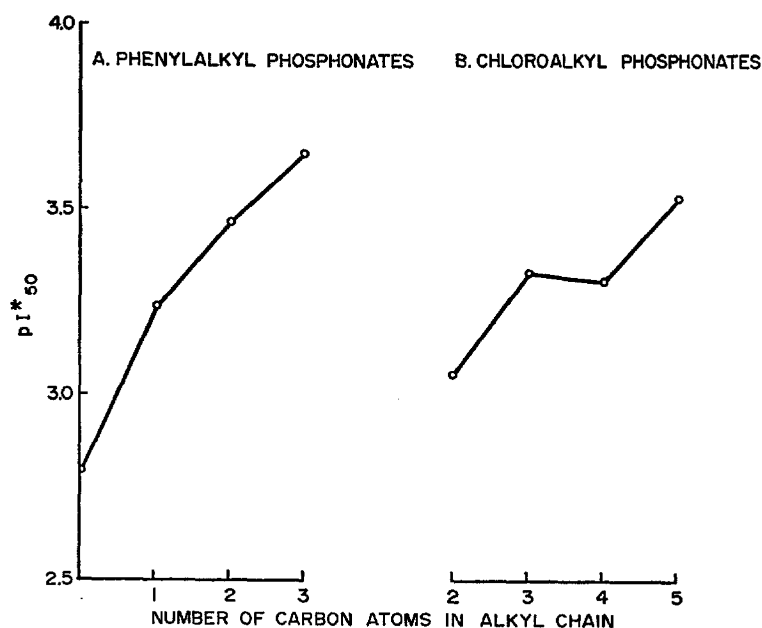

FIG. 4. The effect of the structure of the *p*-nitrophenylethyl phenylalkyl phosphonates (*A*) and the *p*-nitrophenylethyl chloroalkyl phosphonates (*B*) on their ability to inhibit homocytotropic antibody–mediated histamine release. The experiment with the phenylalkyl phosphonates was performed with mast cells sensitized with rat antiovalbumin 6, and the experiment with the chloroalkyl phosphonates was done with cells sensitized with rat anti-*N. brasiliensis*.

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These studies indicate that the phosphonate esters do not react irreversibly on the cells but are acting only on an antigen-antibody–activated esterase.

Effect of Structure on the Activity of the Phosphonate Inhibitors.—The I^*_{50}, the concentration of inhibitor giving 50% inhibition, was obtained for each phosphonate by plotting the per cent inhibition against inhibitor concentration. From this the pI^*_{50}, the negative logarithm of the I^*_{50} was calculated. As previously (4), the * signifies that the time interval over which the inhibitor is acting is unknown. Comparison of the activity of a given series of inhibitors, or of one inhibitor with another, was only made within the same experiment.

Fig. 4, *A* shows that in the aromatic series, the inhibitory power increases regularly in going from the phenylphosphonate to the phenylpropyl phosphonate.

In the chloroalkyl series (Fig. 4, *B*) there is a distinct increase in inhibitory effectiveness of the 3-chloropropyl phosphonate compared to the 2-chloroethyl phosphonate. There is little, if any, demonstrable difference between the 3-chloropropyl and 4-chlorobutyl phosphonates, whereas the 5-chloropentyl phosphonate is distinctly more effective than either.

As seen in Table IV, among the three pentyl phosphonates, the 5-aminopentyl phosphonate is the best inhibitor, the 5-chloropentyl phosphonate, intermediate, and the pentyl phosphonate is the least active.

TABLE IV

Ability of Pentyl Phosphonate, 5-Aminopentyl Phosphonate, and 5-Chloropentyl Phosphonate to Inhibit the Homocytotropic Antibody–Mediated Release of Histamine

Phosphonate	I_{50}*
	M
Pentyl phosphonate	13×10^{-4}
5-aminopentyl phosphonate	2.7×10^{-4}
5-chloropentyl phosphonate	3.5×10^{-4}

DISCUSSION

The results just described demonstrate a clear-cut inhibition of histamine release by the various phosphonate esters. This is in sharp contrast to the equivocal inhibition of the same reaction by diisopropylphosphoflouridate (DFP) found by Perrara and Mongar (20). They reported that 10^{-4} M DFP, an organophosphorus inhibitor with the same mode of action as the phosphonates, gave 10 to 15% inhibition of histamine release, whereas, 10^{-3} M DFP gave a 200% enhancement.

Perrara and Mongar ascribed the enhancement at the higher and inhibition at the lower concentration of DFP to the action of the inhibitor on more than

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one step in the sequence of reactions leading to histamine release. Our results show that the enhancement of antigen-antibody–induced histamine release by certain concentrations of some of the phosphonates is due to an impurity in the phosphonate preparation (Fig. 2 and Table II). In the sliced guinea pig lung system, there was no discernible effect of the impurity present in these same phosphonates (4), and DFP showed none of the puzzling behavior seen in the rat mast cell system (2, 4, 20). It is quite possible that the anomalous behavior of DFP found by Perrara and Mongar is not attributable to a dual action of DFP, as they suggest, but rather to an impurity in their preparation. In this regard, it is of interest that certain samples of DFP have been shown to possess an impurity which is capable of reacting with sulfhydryl-requiring proteolytic enzymes such as ficin (21).

The dose-dependent inactivation of histamine release is only obtained when the phosphonates are present during the addition of antigen; there is a complete lack of effect when these same compounds are added to the mast cells and removed before the addition of antigen. In conjunction with the well known irreversible nature of the inhibition by phosphonates this implies that the phosphonates suppress homocytotropic antibody–mediated histamine release by inhibiting an antigen-antibody–activated esterase. The esterase exists in or on the cell in a phosphonate-resistant precursor form until activated by antigen-antibody interaction. This is the same conclusion reached regarding the esterase involved in antigen-induced release of histamine from sensitized guinea pig lung (4).

The effectiveness of the phenylalkyl phosphonates in inhibiting histamine release from rat peritoneal mast cells increases with increasing chain length; there is also a tendency for the inhibitory activity of the chloroalkyl phosphonates to be enhanced by lengthening the alkyl chain. Chymotrypsin reacts with these phosphonates in essentially the same way (22). The greater effectiveness of the 5-aminopentyl phosphonate than the pentyl phosphonate in inhibiting histamine release is, however, unlike the reaction of these compounds with chymotrypsin (4). Nevertheless, the likenesses are sufficient to suggest that chymotrypsin and the homocytotropic antibody–activated esterase of rat peritoneal mast cells are parazymes; i.e., enzymes which have a similar although not identical specificity (cf. reference 4). How valid this suggestion is, and what if any relation the antigen-antibody–activated esterase bears to the already activated chymotrypsinlike enzyme known to be present in rat peritoneal mast cells (23) must be left for future work to decide (cf. reference 20).

Comparison of the Activity of Phosphonate Esters in the Homocytotropic Antibody–Mediated Reactions of the Rat Mast Cell and Guinea Pig Lung Slice.—The arithmetic dose response curve (Fig. 3, *A* and *B*) found for the action of the phosphonates on the antigen-induced histamine release from rat peritoneal

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mast cells is in distinct contrast to the logarithmic relationship found when these same phosphonates inhibited the sensitized guinea pig lung system (4). The two different kinds of dose response curves imply a difference in the detailed mode of histamine release from the mast cells of the guinea pig lung and the rat peritoneum. In view of the striking variations in their reactivity to chemical histamine release (1, 24) and the morphologic differences demonstrated for the mast cells of the two species (25, 26) this is not unexpected. Alternatively, the form of the dose response curve given by the phosphonates acting on the guinea pig lung reaction might have been determined by possible difficulties of access of the phosphonates or the antigen to the target cell imbedded in the lung tissue. Finally, it should be recalled that even the mediating homocytotropic antibodies in the two species are somewhat different; i.e., the guinea pig antibody is present in antisera in high concentration, is sulfhydryl resistant, and heat stable (27, 28), whereas, the rat antibody is present in trace amounts, is sulfhydryl sensitive, and heat labile (8, 9, 29).

Structural variations of the phosphonates had different effects on their ability to suppress the reaction in the two species. In the guinea pig lung system, the phenylalkyl phosphonates exhibited a sharp peak of activity with benzyl phosphonate; with the rat peritoneal mast cells the inhibitory activity increased progressively with increasing chain length, so that peak activity was observed with the phenylpropyl phosphonate (Fig. 4, *A*). The 5-aminopentyl phosphonate was less inhibitory than the pentyl phosphonate in the sensitized guinea pig lung system, but more inhibitory in the rat mast cell reaction. On the other hand, there is a relatively small difference in the pattern of response of the chloroalkyl phosphonates in the two systems (reference 4 and Fig. 4, *B*).[3]

These differences in the inhibitory pattern of the phosphonates found in the guinea pig and rat systems are much greater than what has been observed in regard to the action of these same compounds on guinea pig and rat C′1a from different species. This is evident in Fig. 1 where the similarity of action of the phosphonates on C′1a from guinea pig, and rat is seen, and also in reference 30, Fig. 2, showing the inhibition of rabbit C′1a. The same sort of species similarity was present when the action of the phosphonates was tested on acetylcholine esterase from rabbit, guinea pig, and human erythrocytes (35).

The enzymatic breakdown of the phosphonates by the guinea pig lung slices (4) or by the rat peritoneal mast cells is negligible so that the variability of the two species in this regard can be discounted. Thus, the differences in the response of the guinea pig lung slices and rat peritoneal mast cells to inhibition by

[3] In the work on the sensitized guinea pig lung system, described in reference 4, an error in the calculation of the concentration of the 5-chloropentyl phosphonate led to a pI_{50}^* which was less than the true pI_{50}^*. When this error was corrected, the pI_{50}^*, 3.4_1, is essentially the same as the pI_{50}^* for the 4-chlorobutyl phosphonate, 3.3_2 determined at the same time in the same system.

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the phosphonates imply that two different antigen-antibody–activated esterases are involved in the homocytotropic antibody–mediated histamine release from the two systems.

Comparison of the Esterase Involved in Homocytotropic Antibody–Mediated Release of Histamine from Rat Mast Cells with Rat C′1a.—The phosphonates give a decidedly different pattern of inhibition when acting on the homocytotropic antibody–mediated release of histamine from rat peritoneal mast cells than when reacting with C′1a of rat or guinea pig complement (Figs. 1 and 4). Among the phenylalkyl phosphonates, the phenyl phosphonate was the most active inhibitor of rat C′1a and the phenylethyl phosphonate was the least (Fig. 1). In the rat mast cell reaction the inhibitory potency increased regularly with increasing distance of the phenyl ring from the phosphorus atom, the phenylpropyl phosphonate being the most effective (Fig. 4, *A*). The inhibitory patterns of the chloroalkyl series in the two reactions were also quite different. The inhibition of C′1a was maximum with the 4-chlorobutyl phosphonate and decreased with the 5-chloropentyl phosphonate. In contrast, the 5-chloropentyl phosphonate was more active in the rat mast cell reaction than the 4-chlorobutyl phosphonate.

As already pointed out, the hydrolysis of the phosphonates by the sensitized or unsensitized mast cell or erythrocyte is negligible. The differences in inhibitory activity of the phosphonates therefore, can not be attributable to differences in the ability of these cells to break down the phosphonates. The present studies also eliminate the possibility that unequal access of the phosphonates to the target cell might account for the differences found, since the effect of the phosphonates was compared on two kinds of cell suspension, the mast cell and the red cell. Thus, the results with the rat peritoneal mast cells lead to the same conclusion as the previous work with guinea pig lung slices; i.e., the antigen-antibody–activated esterase involved in the homocytotropic antibody–mediated release of histamine is not part of the complement system.

SUMMARY

The ability of a number of *p*-nitrophenylethyl, alkyl phenylalkyl, chloroalkyl, and aminoalkyl phosphonates to inhibit the homocytotropic antibody–mediated release of histamine from rat peritoneal mast cells has been tested. The effectiveness of these same phosphonates against the activated first component of rat complement (C′1a) has also been investigated.

The rat mast cell esterase activated by the reaction of antigen and homocytotropic antibody resembles chymotrypsin in its reactivity with the phenylalkyl and chloroalkyl phosphonate, but is unlike this protease in its greater responsiveness to the 5-aminopentyl phosphonate relative to the pentyl phosphonate. The antigen-homocytotropic antibody–activated mast cell esterase and chymotrypsin, thus, appear to be similar, but different enzymes; i.e., they are parazymes (see reference 4, p. 501).

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There are distinct differences in the pattern of inhibition given by the phenylalkyl and aminoalkyl and alkyl phosphonates of the homocytotropic antibody–mediated histamine release from rat peritoneal mast cells and from guinea pig lung slices. On the basis of these differences it is concluded that the esterases activated by the combination of antigen and homocytotropic antibody on the mast cells of the two species are not the same.

The arithmetic dose response curve found for the action of the phosphonates on the antigen-induced histamine release from rat peritoneal mast cells contrasted sharply with the logarithmic relationship found when these same inhibitors acted on the guinea pig lung system. This suggests that in addition to the antigen-antibody–activated esterases being unlike, the detailed mode of histamine release from the mast cells of the guinea pig lung differs from that of the mast cells of the rat peritoneum.

Distinct and large differences were found in the pattern of inhibition of histamine release from rat peritoneal mast cells and of rat C′1a given by the phenylalkyl, and chloroalkyl and alkyl phosphonates implying that esterase activated by the combination of antigen with the sensitized rat peritoneal mast cells is not C′1a. Thus, the results with the peritoneal mast cells lead to the same conclusion as the previous work with guinea pig lung slices; i.e., the antigen-antibody–activated esterase involved in the homocytotropic antibody–mediated release of histamine is not part of the complement system.

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