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HISTAMINE RELEASE BY BEE VENOM PHOSPHOLIPASE A AND MELLITIN IN THE RAT

BY

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Bee venom, first demonstrated to have histamine-releasing properties in animal tissues by Feldberg & Kellaway (1937), contains at least two principles which may be responsible for this effect. Mellitin, a basic protein isolated and characterized by Neumann & Habermann (1954), has pronounced cytolytic activity, which can include mast cell damage. Phospholipase A, another component, is, according to Högberg & Uvnäs (1957, 1960), able to degranulate rat mesentery mast cells *in vitro*, and to release histamine from rat isolated peritoneal fluid mast cells. The haemolytic action of mellitin and phospholipase A has been studied and a marked difference between their mechanism of action been pointed out (Neumann, 1954). Thus mellitin attacks washed red cells without requiring extracellular promoting agents. In contrast, phospholipase A is inactive itself, but readily induces haemolysis when tested on red blood cells in the presence of egg yolk or lecithin. In this instance, the intermediary formation of lysolecithin, a powerful but unspecific cytolytic agent, is responsible for the haemolytic action observed. Under the conditions described by Högberg & Uvnäs (1957, 1960), phospholipase A attacked rat washed mast cells in the absence of extracellular cofactors. They concluded that the primary substrates for this action were phospholipid components of the mast cell membrane, which in this respect would differ fundamentally from the red blood cell membrane whose phospholipid components are believed to be shielded from attack by phospholipase A (Condrea, de Vries & Mager, 1964).

Highly purified preparations of phospholipase A and mellitin from bee venom have been described (Habermann & Neumann, 1957). The present paper reports results of studies on histamine-releasing and mast cell-disrupting properties of these preparations in the rat, and of the effect of the presence of a source of lecithin (egg yolk), on the activity of phospholipase A. An inhibitor of oxidative phosphorylation, 2,4-dinitrophenol, has been shown to inhibit the release of histamine by Compound 48/80, antigen (Rothschild, Vugman & Rocha e Silva, 1961; Diamant & Uvnäs, 1961), curare (Rothschild, 1963) and dextran (Beraldo, Dias da Silva & Lemos Fernandes, 1962) in the rat. These findings have been taken as evidence in favour of the hypothesis that a major step in the mechanism of histamine release depends on mast cell energy metabolism, and bears, in its biochemical aspects, a resemblance to events occurring during the contraction of the muscle fibre (Rothschild, 1963). It was, therefore, considered of interest to verify whether the presence of dinitrophenol affected the activity of phospholipase A and mellitin on rat mast cells.

METHODS

Animals. All experiments were performed on female Wistar rats weighing between 180 and 250 g.

Skin reactions. The hair over the ventral surface was clipped, avoiding irritation to the skin. The animal was lightly anaesthetized with ether, the jugular region exposed, and 0.5 ml. of a 1% solution of Evans Blue in 0.9% saline was injected intravenously. After 5 min, the desired amounts of the test substances, dissolved in 0.05 ml. of saline, as well as saline controls, were injected intradermally. The rats were killed 15 min after the injections and the skin was stripped from the subcutaneous tissue. The extent of the capillary permeability increase, observed on the internal surface of the skin, was estimated by assigning an arbitrarily established scale of values to the intensity and extent of the blueing observed at each site. Inhibitors were injected intraperitoneally, 25 min before the injection of the dye.

Histamine release in vitro. Incubations were performed in Krebs-Ringer phosphate buffer, *p*H 7.3, containing (in mM) NaCl 140, KCl 6, $CaCl_2$ 1.6, $MgCl_2$ 1.5, Na_2HPO_4 10, and, unless otherwise stated, glucose 100 mg/100 ml. Pieces of shaved abdominal skin of rats were excised, rinsed in cold buffer and incubated for 20 min at 37° C, either in buffer alone or in the presence of 2,4-dinitrophenol. The releasing agent was added and incubations continued for another 20 min. Released and bound histamine were estimated by bioassay on the isolated, atropinized guinea-pig ileum. Nonreleased histamine was extracted from the skin samples by grinding in 0.1 N-hydrochloric acid (Feldberg & Talesnik, 1953). Histamine release is expressed as a percentage of the total tissue histamine. After each series of estimations, the presence of spasmogenic material other than histamine was excluded by repeating the assay in the presence of diphenhydramine (5×10^{-7}). Mixed cells from the peritoneal cavity of rats were obtained by flushing the cavity of the exsanguinated animal with 8 ml. of buffer and collecting the cells after brief centrifugation of the suspension. After washing, the sedimented cells were evenly suspended in buffer and added to the incubation mixture. After incubations, performed as described for skin, the flasks were chilled and centrifuged in a refrigerated centrifuge to separate released from cell-bound histamine. Phospholipase A-treated egg yolk, mellitin, as well as phospholipase A itself, exhibited a spasmogenic activity on the ileum which interfered with the assay of released histamine. In the case of the pure enzyme, this activity, although very intense at first contact, showed tachyphylaxis and rapidly became imperceptible. The interference caused by mellitin and phospholipase A-treated egg yolk could be overcome by neglecting the direct estimation of released histamine in the supernatant fluids, and using only the values for histamine in the sedimented residue as a measure of release. This procedure had been previously used (Rothschild, 1962) and found to give a reliable estimate of histamine release.

Morphological alterations of mast cells. Observations were made on pieces of rat mesentery incubated under the same conditions as rat skin. Procedures for fixing, staining and mounting the mesentery spreads were essentially those of Mota & Ishii (1960). Mast cells, microscopically examined at 200-fold magnification, were considered to have been affected by the experimental conditions either when they exhibited granular, metachromatically stained material around their periphery, or when their contours had lost their smooth appearance, becoming irregular and twisted. A total of approximately 300 mast cells was counted in each sample. The extent of the morphological changes induced is expressed as a percentage of altered mast cells.

Haemolytic activity. Oxalated or citrated blood was obtained by heart or venipuncture. After repeated washings with saline, a 5% suspension of red cells in saline was prepared. After 30 min incubation at 37° C with the test substances, the cells were centrifuged and the extent of haemolysis was estimated in the supernatant fluid, by adding one drop of 10% potassium ferricyanide to it and measuring the amount of methaemoglobin formed, using a Klett photocolorimeter with a green filter.

Drugs. Phospholipase A from bee venom was obtained through the courtesy of Professor E. Habermann (Wuerzburg, Germany). It had been purified by fractionation on Sephadex (Habermann, 1963), followed by ion-exchange chromatography (Habermann & Neumann, 1957). Stock solutions of the enzyme in saline were stored in the frozen state. Compound 48/80 was obtained through the courtesy of Dr J. J. Burns (Wellcome Laboratories, Tuckahoe, N.Y.). Mellitin, purified by chromatography, was obtained through the courtesy of Professor E. Habermann. Hen's fresh egg yolk was used as a source of phospholipid substrates. Lysophosphatides were produced by incubating fresh egg yolk, diluted to 40% in 0.9% saline, with 100 μg/ml. of phospholipase A for 30 min at 37° C. Diphenhydramine hydrochloride (Benadryl,

Parke Davis), bromolysergic acid diethylamide (BOL 148, Sandoz, kindly supplied by Dr I. Mota, São Paulo) and 2,4-dinitrophenol (Bayer) were also used.

RESULTS

Bee venom phospholipase A, as well as mellitin and Compound 48/80, promoted localized blueing of the skin at the site of intradermal injections into rats previously treated with Evans Blue (Table 1). The threshold for the response to phospholipase A was between

TABLE 1

EFFECT OF HISTAMINE AND 5-HYDROXYTRYPTAMINE INHIBITORS ON THE CHANGES OF PERMEABILITY ELICITED BY PHOSPHOLIPASE A, MELLITIN AND COMPOUND 48/80 IN THE CAPILLARIES OF RAT SKIN

The substances to be tested as well as saline controls were injected intradermally into assigned sites of the experimental animals. Each drug was tested in four normal animals, in three animals which had been treated with diphenhydramine, in two which had received bromolysergic acid diethylamide (EOL 148) and in four animals which had received both drugs. ++++ to + refer to relative intensity of the blueing reaction; − means no reaction

Drug	Skin permeability change after treatment with: None	Diphenhydramine (25 mg/kg)	BOL 148 (2 mg/kg)	Diphenhydramine and EOL 148
Phospholipase A (1 μg)	++++	+++	+++	−
	++++	++	++	−
	+++	++		−
	++++			−
Mellitin (1 μg)	+++	++	++	−
	+++	+	++	−
	++++	++		−
	+++			−
Compound 48/80 (1 μg)	++++	+++	++	−
	+++	+	++	−
	++++	++		+
	+++			−
Saline (0·05 ml.)	±	±	−	−
	−	−	−	−
	+	−		−
	−			−

0.001 and 0.01 μg. Table 1 shows the effect of previous treatment with an antihistamine (diphenhydramine), an anti-5-hydroxytryptamine drug (bromolysergic acid diethylamide), or a combination of both agents. It can be seen that, while inhibition was incomplete when either diphenhydramine or bromolysergic acid diethylamide alone was used, complete block of the responses to either of the three test substances was always obtained when the two drugs were administered together.

Phospholipase A was able to release part of the bound histamine of rat skin. As shown in Table 2, the amounts of histamine liberated were of the same order as those freed after treatment with a similar dose of Compound 48/80. The mechanism of the releasing action of either drug appeared to be different. Thus the action of Compound 48/80 was strongly repressed in dinitrophenol-treated skin while that of phospholipase A was not significantly affected. An additional observation, unrelated to the main subject of this work but nevertheless interesting, was made during these experiments. It concerns the effect of dinitrophenol on the so-called spontaneous release of histamine; such a release is usually

TABLE 2

RELEASE OF HISTAMINE FROM RAT SKIN *IN VITRO* BY PHOSPHOLIPASE A AND BY COMPOUND 48/80 AND THE EFFECT OF 2,4-DINITROPHENOL

Dinitrophenol-treated samples were left in contact with the inhibitor (3×10^{-4} M) for 20 min at 37° C before the addition of the releasing agent. Incubations were performed in glucose-free media. Values are the means and standard errors of six experiments. Figures for net release were obtained after deducting values for histamine release observed in blanks incubated in the absence of the releasing agent. Significance (for the effect of dinitrophenol) was determined by Student's *t*-test

	Histamine released (%)		
Releasing agent	Controls	Dinitrophenol	*P*
None	14·8±1·9	7·3±1·4	<0·05
Phospholipase A (20 μg/ml.)	21·9±1·8	16·6±2·8	Not significant
Net release	7·1	9·3	
Compound 48/80 (20 μg/ml.)	22·6±2·6	8·8±1·1	<0·005
Net release	7·8	1·5	

observed when rat isolated tissues are incubated in the absence of added histamine-releasing drugs. As shown in Table 2, 3×10^{-4} M-dinitrophenol significantly decreased the amounts of histamine released during the incubation of pieces of untreated rat skin in Krebs-Ringer phosphate buffer.

The ability to degranulate tissue mast cells *in vitro* is a property of most histamine-releasing drugs active in the rat. Phospholipase A was an apparent exception to this rule. Table 3 shows that no significant changes in mast cell appearance became apparent follow-

TABLE 3

IN VITRO EFFECTS OF PHOSPHOLIPASE A-TREATED EGG YOLK, COMPOUND 48/80 AND MELLITIN ON THE DEGRANULATION OF RAT MESENTERY MAST CELLS AND THE RELEASE OF HISTAMINE FROM RAT PERITONEAL FLUID MAST CELLS

Values for mast cell degranulation are results of determinations made on pieces of mesentery from two animals. Values for histamine release are means with standard errors; they are presented after deduction of the release obtained in corresponding blanks incubated in buffer or untreated egg yolk; release in blanks was always less than 10% of the total histamine present. Figures within parentheses indicate the number of experiments performed

Treatment	Mesentery mast cells showing morphological alterations (%)	Histamine released from peritoneal fluid cells (%)
None	1·1, 4.0	—
Phospholipase A (50 μg/ml.)	11·8, 2·3	12·0±5·6 (5)
Phospholipase A (10 μg/ml.)	2·5, 0	10·5±3·3 (14)
Egg yolk (4%)	1·1, 2·3	—
Phospholipase A-treated egg yolk (4%)	59·0, 70·7	84·4±7·5 (5)
Compound 48/80 (5 μg/ml.)	98·0, 99·0	78·9±2·7 (12)
Mellitin (10 μg/ml.)	97·0, 92·0	75·4±9·4 (5)

ing the incubation of pieces of rat mesentery with 10 to 50 μg/ml. of phospholipase A. In contrast, the product of the incubation of phospholipase A with a diluted suspension of egg yolk induced morphological alterations in more than 50% of the cells examined. Controls, incubated with untreated egg yolk, did not show these changes. Mellitin was also quite active in promoting mast cell alterations.

It was considered of interest to study the histamine-releasing action of phospholipase A in a system free of extracellular phospholipids. Washed mast cells, obtained from the peritoneal cavity of rats, readily release their histamine when incubated with histamine

liberators. Table 3 shows that 10 μg/ml. of phospholipase A released only 10% of the histamine from these cells. This effect was not significantly increased when the concentration of the enzyme was raised to 50 μg/ml., or, as tried in two experiments, to 100 and 250 μg/ml., respectively. These results could have been due to the absence of activating factors. Activating effects of sulphydryl compounds on snake venom phospholipase A have been indicated by Gosh & Sarkar (1956); a similar effect of serum albumin on bee venom phospholipase A has been described by Habermann (1957). Accordingly, experiments were performed in the presence of cysteine (100 μg/ml.) and bovine serum albumin (Armour's Fraction IV, 1 mg/ml.), respectively. No evidence of an enhanced release of histamine due to these treatments could be obtained. Rat serum, present in 1 : 4 dilution, also failed to affect the action of phospholipase A. An extensive release of histamine could be produced, however when lysophosphatides, produced by incubating phospholipase A with fresh egg yolk, were added to the mast cell suspensions. The amounts of histamine thus freed averaged 84% of the total amine present. Controls, made with untreated egg yolk, showed no histamine release. Additional results in Table 3 show that, in agreement with their marked action on mesentery mast cells, Compound 48/80 released 79% and mellitin 75% of the histamine of mast cells isolated from peritoneal fluid. Table 4 shows that the

TABLE 4

EFFECT OF 2,4-DINITROPHENOL ON THE *IN VITRO* RELEASE OF HISTAMINE FROM RAT PERITONEAL FLUID MAST CELLS BY PHOSPHOLIPASE A-TREATED EGG YOLK, COMPOUND 48/80 AND MELLITIN

Values are means of three experiments. Incubation conditions were as described in Table 2

Treatment	Histamine released (%)		
	Controls	Dinitrophenol	Dinitrophenol and glucose
Phospholipase A-treated egg yolk (4%)	91	93	92
Compound 48/80 (5 μg/ml.)	79	14	80
Mellitin (10 μg/ml.)	91	94	95

histamine-releasing action of phospholipase A-treated egg yolk as well as that of mellitin was not decreased by dinitrophenol. In contrast, the release due to Compound 48/80 was strongly depressed. In similarity with results previously obtained in the rat isolated diaphragm preparation (Rothschild *et al.*, 1961), glucose was able to overcome fully this inhibitory effect of dinitrophenol.

Rat washed erythrocytes were not sensitive to the action 25 μg/ml. of phospholipase A, but were totally haemolysed by the product of the incubation of the enzyme with egg yolk; this action, again, was not inhibited by 3×10^{-4} M-dinitrophenol. Similar results were obtained with the red cells from other species (cat, mouse, dog, man, sheep and guinea-pig).

DISCUSSION

Bee venom phospholipase A and mellitin, as well as Compound 48/80, increased skin capillary permeability in the rat. This effect was apparently due to the release of histamine and 5-hydroxytryptamine, since it was fully blocked in animals previously treated with inhibitors of the vascular effects of these amines. The histamine-releasing activity of phospholipase A in rat skin was confirmed *in vitro*; the percentage of histamine released by

phospholipase A in these experiments was comparable to that liberated by Compound 48/80. A marked difference was, however, shown when the action of phospholipase A on the degranulation of rat isolated mesentery mast cells or the release of histamine from washed peritoneal fluid mast cells was compared with that of Compound 48/80. The latter was highly effective in both systems; in contrast, phospholipase A was inactive on mesentery mast cells, and only slightly active on isolated peritoneal fluid mast cells. The apparent discrepancy between the results obtained in rat skin on the one hand, and in isolated mesentery or peritoneal fluid mast cells on the other, can be explained if it is accepted that phospholipase A, although incapable of attacking the mast cell membrane, is nevertheless able to promote the formation of surface-active, cytolytic agents of the lysolecithin type, by hydrolysing phospholipid constituents of body fluids. Substrates for such an action could be expected to be present in lymph (Feldberg, Holden & Kellaway, 1938), held in the extracellular spaces of skin, but to be virtually absent from washed samples of excised mesentery or isolated mast cell suspensions. The marked histamine-releasing and mast cell-degranulating activity of lysolecithin, produced by incubating phospholipase A with a source of lecithin like egg yolk, lends supporting evidence for these conclusions.

Dinitrophenol, an inhibitor of oxidative phosphorylation, blocked the histamine-releasing action of Compound 48/80, but did not affect histamine release from rat isolated skin by phospholipase A. The actions of egg yolk lysolecithin or mellitin on washed mast cells or erythrocytes were also not affected by this inhibitor. These results are not surprising, if it is accepted that, by analogy with their haemolytic activities (Robinson, 1961; Habermann & Neumann, 1957), the actions of lysolecithin and mellitin on mast cells are a consequence of their high surface-active properties. Substances having such activity can be regarded as almost unspecific cytolytic agents, whose histamine-releasing effects would have little of the precise biochemical requirements shown (Mota & Ishii, 1960; Rothschild, 1963) to condition the action of Compound 48/80 or other dinitrophenol-sensitive histamine-releasing stimuli.

The concept of an activation of tissue phospholipase A during antigen-antibody reactions in animal tissues (Feldberg & Kellaway, 1938) has been recently revived (Högberg & Uvnäs, 1957, 1960; Uvnäs, 1962). Although direct experimental evidence for this hypothesis is still lacking, it is attractive because it gives a rational explanation for observations (Brocklehurst, 1956; Chakravarty, Högberg & Uvnäs, 1959) which show that, during anaphylaxis, a slow-reacting, smooth muscle stimulating substance (SRS) is formed. This principle seems to be a fatty acid (Chakravarty *et al.*, 1959), possibly identical with one of the products of the action of phospholipase A on tissue lecithin or an equivalent substrate. Mast cells are involved in the anaphylactic reaction in the rat (Mota & Ishii, 1960), and it has been postulated (Uvnäs, 1962) that the slow reacting substance arises from the enzymatic breakdown of phospholipids of the membrane of the mast cell. Since, as the present work shows, mast cell membranes are resistant to the direct action of phospholipase A, it is possible that the slow reacting substance does not come from mast cells. Support for this possibility is provided by results (Rapp, 1961) which show that the slow reacting substance was not formed during the action of antigen on rat isolated sensitized peritoneal fluid mast cells.

It was interesting to note in our experiments on rat isolated skin that the release of histamine observed in the absence of added releasing agents could be partially blocked by

dinitrophenol. This result indicates that the so-called spontaneous loss of histamine which is usually observed during the incubation of rat tissues in buffered media may not be merely due to diffusion or loss of histamine from damaged parts of the tissue. In view of its sensitivity to dinitrophenol, it appears that part of this process has the same dependence on cell metabolism as has the action of Compound 48/80 on the mast cells of this species.

SUMMARY

1. The activities of purified preparations of bee venom phospholipase A and mellitin on rat skin capillary permeability, histamine release and mast cell degranulation have been examined and compared with those of Compound 48/80.

2. All three compounds increased capillary permeability; this effect was blocked by antagonists of histamine and 5-hydroxytryptamine and is probably due to the release of these amines.

3. Phospholipase A as well as Compound 48/80 released histamine from rat skin *in vitro*; in contrast to 48/80, the enzyme was also active on skin previously treated with 2,4-dinitrophenol in the absence of glucose. This suggests that rat mast cell metabolic activity, which is important for the action of 48/80, is not a prerequisite for the histamine-releasing action of phospholipase A.

4. Phospholipase A did not degranulate rat mesentery mast cells *in vitro* and only released small amounts of histamine from rat washed peritoneal fluid cell suspensions. Extensive mast cell degranulation and histamine release were, however, observed with mellitin or Compound 48/80.

5. Lysolecithin, formed by incubating hen's fresh egg yolk with phospholipase A, readily attacked mesentery or peritoneal fluid mast cells causing morphological alterations and loss of histamine. These effects were not inhibited by dinitrophenol.

6. Phospholipase A did not haemolyse washed erythrocytes of the rat or other species; in contrast, lysolecithin was strongly haemolytic and its activity was not influenced by dinitrophenol.

7. The results obtained indicate, by analogy with its relationship to red blood cells, that phospholipase A has no, or only a slight, direct action on rat mast cells. Its histamine- and 5-hydroxytryptamine-releasing action on rat skin *in vivo* seems to be indirect and is probably a consequence of the enzymatic cleavage of tissue fluid phospholipids. This could lead to the appearance of nonspecific, surface-active cytolytic agents similar to egg yolk lysolecithin.

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REFERENCES

BERALDO, W. T., DIAS DA SILVA, W. & LEMOS FERNANDES, A. D. (1962). Inhibitory effects of carbohydrates on histamine release and mast cell disruption by dextran. *Brit. J. Pharmacol.*, **19**, 405–413.

BROCKLEHURST, W. E. (1956). A slow reacting substance in anaphylaxis " SRS-A." In *Ciba Symposium on Histamine*, pp. 175–179. London: Churchill.

CHAKRAVARTY, N. R., HÖGBERG, B. & UVNÄS, B. (1959). Mechanism of the release by compound 48/80 of histamine and of a lipid soluble smooth muscle stimulating principle (" SRS "). *Acta physiol. scand.*, **45**, 255–270.

CONDREA, E., DE VRIES, A. & MAGER, J. (1964). Hemolysis and splitting of human erythrocyte phospholipids by snake venoms. *Biochim. biophys. Acta (Amst.)*, **84**, 60–73.

DIAMANT, B. & UVNÄS, B. (1961). Evidence for energy-requiring processes in histamine release and mast cell degranulation in rat tissue induced by compound 48/80. *Acta physiol. scand.*, **53**, 315–329.

FELDBERG, W., HOLDEN, H. F. & KELLAWAY, C. H. (1938). The formation of lysocithin and of a muscle-stimulating substance by snake venoms. *J. Physiol. (Lond.)*, **94**, 232–248.

FELDBERG, W. & KELLAWAY, C. H. (1937). Liberation of histamine and its role in the symptomatology of bee venom poisoning. *Aust. J. exp. Biol. med. Sci.*, **15**, 461–473.

FELDBERG, W. & KELLAWAY, C. H. (1938). Liberation of histamine and formation of lysocithin-like substances by cobra venom. *J. Physiol. (Lond.)*, **94**, 187–226.

FELDBERG, W. & TALESNIK, J. (1953). Reduction of tissue histamine by compound 48/80. *J. Physiol. (Lond.)*, **120**, 550–568.

GOSH, B. N. & SARKAR, N. K. (1956). Active principles of snake venoms. In *Venoms*, publ. 44, pp. 189–196. Washington, D.C.: Amer. Ass. Adv. Sci.

HABERMANN, E. (1957). Manometrische Bestimmung von Phospholipase A. *Biochem. Z.*, **328**, 474–484.

HABERMANN, E. (1963). Recent studies on hymenoptera venoms. *Biochem. Pharmacol.*, suppl. **12**, 187.

HABERMANN, E. & NEUMANN, W. P. (1957). Reinigung der Phospholipase A des Bienengiftes. *Biochem Z.*, **328**, 465–473.

HÖGBERG, B. & UVNÄS, B. (1957). The mechanism of the disruption of mast cells produced by compound 48/80. *Acta physiol. scand.*, **41**, 345–369.

HÖGBERG, B. & UVNÄS, B. (1960). Further observations on the disruption of rat mesentery mast cells caused by compound 48/80, lecithinase A, decylamine and antigen-antibody reaction. *Acta physiol. scand.*, **48**, 133–145.

MOTA, I. & ISHII, T. (1960). Inhibition of mast cell disruption and histamine release in rat anaphylaxis *in vitro*. Comparison with compound 48/80. *Brit. J. Pharmacol.*, **15**, 82–87.

NEUMANN, W. (1954). Neuere Untersuchungen über die Giftstoffe von Bienen und Schlangen. *Naturwissenschaften*, **14**, 322–326.

NEUMANN, W. & HABERMANN, E. (1954). Beitrage zur Charakterisierung der Wirkstoffe des Bienengiftes. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharm.*, **222**, 367–387.

RAPP, H. J. (1961). Release of a slow reacting substance (SRS) in the peritoneal cavity of rats by antigen-antibody interaction. *J. Physiol. (Lond.)*, **158**, 207–208*P*.

ROBINSON, N. (1961). Lysolecithin. *J. Pharm. Pharmacol.*, **13**, 321–354.

ROTHSCHILD, A. M. (1962). Effect of catecholamines and their chloro- analogues on the *in vitro* release of histamine from rat peritoneal fluid cells. *Biochem. Pharmacol.*, **11**, 97–98.

ROTHSCHILD, A. M. (1963). Metabolic aspects of cellular response to histamine releasing stimuli. *Biochem. Pharmacol.*, suppl. **12**, 212.

ROTHSCHILD, A. M., VUGMAN, I. & ROCHA E SILVA, M. (1961). Metabolic studies on the release of histamine by compound 48/80 in the rat diaphragm. *Biochem. Pharmacol.*, **7**, 248–255.

UVNÄS, B. (1962). Mechanism of histamine release in mast cells. *Ann. N.Y. Acad. Sci.*, **103**, 278–284.