REVIEW ARTICLE

THE MECHANISM OF HISTAMINE LIBERATION

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THEORIES have to be modified from time to time as fresh experimental data emerge. Recent years have brought new observations as to the localisation and the chemical binding of histamine in the tissues. Synthetic and biologically occurring histamine liberators constitute a new group of more or less specific substances and compounds, the histamine releasing properties of which were, until only a few years ago, completely unknown. The data available today necessitate reconsideration of the validity of existing theories on the mechanism of histamine liberation.

Localisation of Histamine

Histamine is present in virtually all mammalian tissues, but it is very unevenly distributed. There are also extreme variations between different species. For instance, in some species the liver is very rich in histamine (rabbit, dog, horse); in some, histamine is found in high concentrations in the lungs (guinea pig, cattle, horse); other animals have high contents of histamine in the skin (rat, cat), and so on. (For further details of the histamine distribution in the body, see Feldberg[1].)

Riley and West[2] reported a close quantitative correlation of the histamine content of a tissue and the density of its mast cell population. Mast cell tumours (in dogs) were observed to have an extremely high content of histamine (up to 1290 μg./g. of tissue[3]). The conclusion of Riley and West that mast cells store histamine has since been confirmed in various ways and is now generally accepted. In recent experiments Schayer[4] was able to demonstrate the accumulation of radioactive histamine in rat mast cells when radioactive histidine was added to the cells *in vitro*, thus indicating the intracellular formation of histamine.

But a satisfactory quantitative correlation of the histamine content and the mast cell population does not exist in all tissues. The gastrointestinal tract, not least the stomach mucosa, has a rather high histamine content but relatively few mast cells. As regards the skin, histamine is reported to occur in the corium, a layer which is said to contain no mast cells. The tissue histamine situated outside the mast cells does not seem to be released by the common histamine liberators.

Even though, for reasons which are quite obscure, histamine may occur outside the mast cells, most of it is localised in them. Of special interest in this context, is the fact that the readily mobilisable histamine in the body is localised in the mast cells. The present discussion will therefore be confined to the liberation of histamine from these cells.

The intracellular distribution of histamine has been studied by differential centrifugation of mast cells. In such experiments the bulk of the

histamine has been recovered from the granular fractions[5]. According to Blaschko[6] the enzymes which produce amines—among them histamine—occur in the cell sap. Histamine should accordingly be formed in the cell sap and then stored in the cell granules. This means that the formed histamine has to pass in through the granular membrane. Whether, under physiological conditions, histamine also passes from the granules into the cell sap, and further through the mast cell membrane into the surrounding tissues, is not known and, in my view, is by no means certain. The matter will be discussed further later in this review.

The mast cell histamine occurs, so to speak, in a double parcel, a large one—the mast cell—containing many small ones—the granules. Both of the parcels are enveloped by membranes with permeability properties of which our knowledge is very hazy and fragmentary. An acceptable theory for the mechanism of histamine release has to explain the passage of histamine through two membranes which seem normally to be impermeable to free histamine.

How is Histamine Chemically Bound in the Cell?

It has been suggested that histamine occurs in the tissue rather firmly bound to proteins or lipoproteins[7]. When these compounds are broken down by proteases or other enzymes the histamine should be set free. However, today there are grounds for believing that this is not the case. How the histamine which is found outside the mast cells is anchored to the tissue is completely unknown, but about the mast cell histamine we have at least some suggestive evidence. McIntire, White and Sproull[8] showed that histamine was readily released from the tissue by treatment with ice-cold trichloroacetic acid, distilled water, cold acetone, acid alcohol, repeated freezing and thawing, and so on. In other words histamine can be removed from a tissue by relatively mild procedures which do not suffice to break firm chemical bonds. Disruption of the anatomical structures or of the permeability barriers of the mast cell apparently suffices to cause a release of the intracellular histamine. Some of these procedures mentioned seem to rule out activation of enzyme mechanism as the cause of histamine release. It seems more reasonable to conclude, as did McIntire, that histamine is held in the mast cell by fairly weak ionic linkages. Such linkages would break rather easily, for example even with shifts in the ionic equilibrium within the granules.

The mast cell granules contain heparin, which is a mucopolysacharide with acid groups. Presumably there are, in the granules, many other compounds with acid groups; hence it is not difficult to conceive of histamine as occurring in the cell in loose ionic linkage with various acid groups.

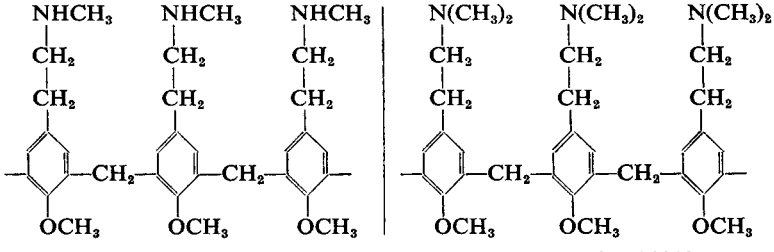
When is Histamine Liberated?

To Sir Henry Dale the credit is due for having drawn attention to the possible role of histamine as an active agent in anaphylactic reactions. The release of histamine with antigen-antibody reactions in sensitised

tissues has since been studied by Dale, by Feldberg, by Dragstedt and their associates as well as numerous others.

Many histamine liberators have been discovered in the last ten to fifteen years. MacIntosh and Paton[9] described a number of amines, amidines, guanines, guanidines and other organic bases, all of which were more or less potent histamine liberators. Since then the list of substances and compounds capable of histamine release has increased continually. To the group belong many drugs, and several others have been isolated from biological sources, as for instance sea anemone, jellyfish, Ascaris organisms, caterpillars, etc. An extensive list of known histamine liberators is given in Paton's excellent review[10].

Two synthetic liberators require further mention for the purposes of the discussion below. Compound 48/80 is a polymer amine, a condensation product between formaldehyde and *p*-methoxy-phenethyl-methylamine. It is a very potent histamine liberator in some animals like the rat, cat and dog, but fairly inactive in the guinea pig. For reasons mentioned later on, a polymer amine was synthesised that differed from compound 48/80 in that it had two methyl groups linked to the nitrogen. This polymer tertiary amine was also a potent histamine liberator. The two polymers are shown in Figure 1.



Compound 48/80 Compound PK3010*aa*

FIG. 1. Polymer histamine liberators.

Mast Cell Changes on Histamine Release

When histamine is released from a tissue as the result either of an antigen-antibody reaction or of the action of a histamine liberator, the mast cells in the tissue undergo profound morphological changes. The cells become degranulated and more or less lose their staining characteristics for basic dyes.

The mast cell changes have mostly been studied in rat tissues. In rats treated repeatedly with 48/80 the cutaneous mast cells disappear parallel with a decline of the cutaneous histamine content to extremely low levels. For a period of some weeks there is then a slow continuous increase of the cutaneous histamine, and at the same time the mast cells return[11].

When mast cells of rat mesentery are incubated *in vitro* with compound 48/80, their granules are seen to be spread around the cells, which have an "exploded" appearance. However, it is not yet clear whether this degranulation means destruction of the cell membrane and devitalisation

of the mast cell, or merely a temporary increase in the membrane permeability consistent with survival of the cell. Recent observations rather suggest that the histamine-containing granules may be discharged from the mast cells, leaving the cell capable of renewing its histamine store. Possibly all degrees may occur, from irreversible destruction to slight permeability changes in the cell membrane, according to the intensity of the liberation process. The matter will be further discussed later.

Theories of the Histamine Liberation Mechanism

Knowing a little about the localisation and chemical status of histamine in the tissue, and the circumstances under which it may be released from its storage therein, we can now proceed to discuss some of the theories propounded to explain the mechanism by which it is liberated.

Various enzyme theories have been suggested.

Protease Theories

Various snake venoms release histamine from perfused tissues. Since such venoms are observed to contain trypsin and the latter produced, in animals, a shock-like syndrome on intravenous administration, Rocha e Silva[7] advanced a protease theory to explain the histamine release in anaphylactic shock. He showed that the proteolytic enzymes papain and trypsin were able to release histamine from perfused tissues, and from leucocytes. Further, Ungar[12] reported that antigen-antibody reactions caused a rise in the fibrinolysin activity in the blood serum of guinea pigs concomitantly with the histamine release. Both processes were claimed to be inhibited by soy bean trypsin inhibitor. Increased protease activity was also induced by compound 48/80 and other histamine liberators. The histamine release was consignificantly attributed to the activation of proteases which released histamine by splitting it from the polypeptides. Ungar's latest modification of his theory is somewhat complicated; it involves kinases in the blood and cells that transform proactivators to activators, which in turn transform protease precursors to active proteases, the latter then attacking polypeptides to which histamine is supposed to be attached.

The protease theories have several weak points. The histamine releasing activity of trypsin is weak, and fibrinolysin—even in high concentrations—is not able to release histamine[13,14]. There is no satisfactory quantitative or temporal relation between the protease activity and the histamine release[15]. Lastly we have no evidence whatsoever that histamine occurs in the mast cells—the locus from which it is released—linked to proteins by bonds which r quire proteases for their dissolution. On the contrary, experimental evidence militates against the existence of a firm binding of histamine.

It has to be remembered that the protease theories were propounded before the localisation of histamine in the mast cells had been discovered. The survival of a modified protease theory therefore seems to depend on the ability of proteolytic enzymes to degranulate the mast cells. As will

be seen from Table I, both trypsin and fibrinolysin, even in high concentrations, lack this ability when tested on rat mast cells *in vitro*. In my view, this is an additional argument against the validity of the protease theories.

The Lysolecithin Theory

The strongly haemolytic snake venoms from, for example, *Naja naja* and *Denisonia superba* possess histamine liberating properties. The haemolytic action is due to the presence of lecithinase A in the venoms. Lecithinase A attacks lecithin causing the formation of lysolecithin, which has a haemolytic action. Besides causing haemolysis, lysolecithin is

TABLE I

THE EFFECT OF VARIOUS ENZYMES ON THE MAST CELL MEMBRANE

Enzyme	Max. conc. used μg. or units/ml.	Disruption per cent
Acetylcholinesterase	400	0
α and β Amylases	500	0
Pectinesterase	500	0
Hyaluronidase	20 I.U.	0
β-Glucuronidase	1680 Fishnam units	0
Hexokinase	500	0
Cozymase	400	0
Lipase (pancreas)	400	0
Lecithinase A (bee venom)	200	100
Malic dehydrogenase	200 U.	0
Trypsin	2500	10
Carboxypeptidase	400	0
Thrombin (1)	19 U.	2
Fibrinogen (2)	600	5
1 + 2	—	0
Plasminogen (3)	1 ml.	0
1 + 2 + 3	—	20
Streptokinase (4)	1000 U.	10
1 + 2 + 3 + 4	—	10
4 + rat serum	+0·4 ml.	0
Pancreatin	1000	0
Urease	500	0
Ribonuclease	1000	0
Desoxyribonuclease	1000	0
Uricase	500	0
Phosphatase, alkaline	500	10
Phosphodiesterase (snake venom)	500	0
ATP-ase	100	0
Cytochrome C	500	0
Carbonic anhydrase	500	0

capable of releasing histamine. It was therefore suggested that histamine might be linked to lipoproteins, from which it could be released by the lytic action of lysolecithin[15].

The lysolecithin theory has not aroused any great enthusiasm, presumably because no experimental evidence was presented to support the necessary assumption that histamine occurred in the tissue bound to lipoproteins sensitive to the action of lysolecithin. However, since the discovery of the histamine-bearing role of the mast cells, the validity of a cytolytic theory warrants attention. As will be seen in Table I, lecithinase A is the only enzyme among 25 tested which is capable of disrupting mast cells in the rat mesentery[16]. Lecithinase A prepared from bee venom or various snake venoms is highly active, only a few micrograms being required to cause total disruption of the mast cells.

The histamine release produced by some snake venoms might therefore

be ascribed to the lecithinase activity of the venoms. Yet, when tested on perfused cat paw, even very active lecithinase preparations were able to release only negligible amounts of histamine (unpublished observations). The reason for this discrepancy between the action of lecithinase A on rat mast cells *in vitro* and on perfused cat paw is not clear. The inability of lecithinase A to release histamine in the cat paw might depend on inadequate experimental conditions, e.g. the lecithinase not reaching its target, the mast cells. After injection of lecithinase the paws become very oedematous, and the perfusion tends to decrease and cease. Possibly the lecithinase does not pass in through the capillary walls. As will be discussed later, the ineffectiveness of lecithinase administered into the circulation does not invalidate a modified lecithinase theory.

Other Enzyme Theories

The histamine release in guinea pig tissues as a result of antigen-antibody reactions has been ascribed to an energy-requiring enzymatic process, since the histamine release is reduced by oxygen lack, and by the presence of iodo-acetic acid and some other metabolic inhibitors[17]. It was also observed to be inhibited by previous heating of the sensitised tissue to 43 to 44°[18]. All of these observations are consistent with the idea of an enzymatic release of histamine. The action of 48/80 and octylamine, on the other hand, was attributed to a different mechanism. The release of histamine produced by these agents in guinea pig tissue was found to be enhanced by oxygen lack and by iodoacetic acid.

On the other hand, Junqueira and Beiguelman[19] claimed that various enzyme inhibitors blocked the disrupting action of 48/80 on *rat mesentery mast cells*. Special attention was given to the inhibitory action or SH-blocking compounds (*p*-Cl-Hg-benzoate, *O*-iodosobenzoate, iodoacetate). There is no doubt from their figures that these enzyme inhibitors prevent degranulation of the mast cells, the implication being that the action of 48/80 is mediated by an enzymatic mechanism. However, in my view the concentrations of the inhibitors used are too high to guarantee specific blocking of SH-groups, and the experiments therefore do not yield much information about the types of enzymes involved.

The Displacement Theory

Histamine, as mentioned above, is a base that is thought to be loosely linked to acid groups in the intracellular granules; and most synthetic histamine liberators are organic bases, more or less lipid soluble. It seemed plausible to assume that such substances liberated histamine by penetrating the cell and granular membranes and, once inside the granules, simply replaced the histamine[9]. This simple, and hence attractive, hypothesis might explain the histamine release produced experimentally by some organic bases, especially *in vitro* but also *in vivo* when high doses are used or required to produce a release, as is the case with monoamines and also with compound 48/80 in guinea pigs. It is doubtful, however, whether the histamine liberation observed clinically or experimentally even after minute doses of various substances can be explained simply on

an ion exchange basis. Several arguments can in fact be advanced against the displacement theory to which Paton[10] recently subscribed, as being valid for the action of 48/80.

The displacement theory postulates that the liberating agent penetrates the mast cell membrane and enters the granules. As far as I am aware, no such passage of, for example, 48/80 has yet been demonstrated. To me, the well-known fact that one molecule of 48/80 releases several molecules of histamine appears to be a serious obstacle to a displacement theory. In a perfused cat paw, for instance, 10 μg. 48/80 is able to release up to 75 μg. of histamine. Since 48/80 injected into the tibial artery must become widely spread throughout the tissues of the paw, the concentration

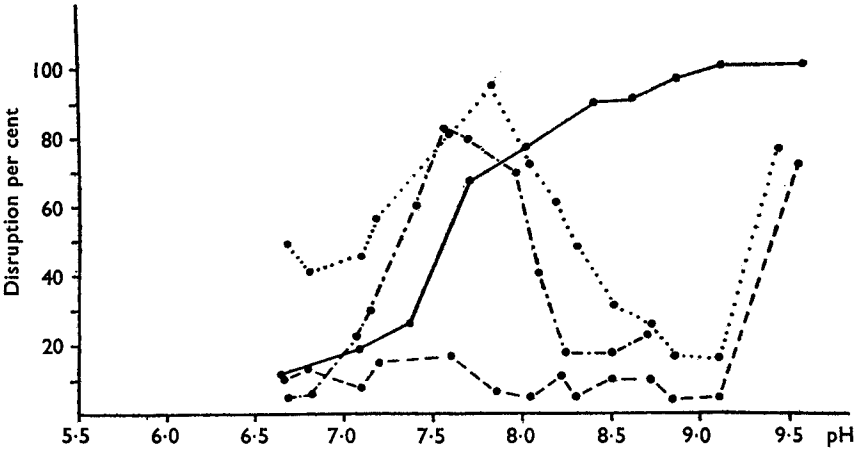


FIG. 2. The influence of pH on the disruptive action of 48/80 on rat mesentery mast cells. Barbitone buffer. · · · 48/80 0·5 μg./ml., – · – Pk3010*aa* 1 μg./ml., —— decylamine 10 μg./ml., – – – control.

of 48/80 around the mast cells will of necessity be very low. To explain the release of histamine as a simple ion exchange between 48/80 and histamine seems rather difficult.

There are other counter-arguments. Compound 48/80 is an amine and its lipid solubility, and hence its ability to penetrate the cell membrane, should increase with decreasing ionisation. In other words, the more alkaline the medium the higher should be the disrupting action of 48/80 on the mast cells. However, as shown in Figure 2 (Högberg and Uvnäs, 1957, unpublished observations), this is not the case. Pieces of rat mesentery were incubated with 48/80 at various pH values. The solution was buffered with either 10 per cent phosphate or 10 per cent barbitone buffer. The disruptive action of 48/80 on the mast cells showed a peak around pH 7·8 and reached low values on both sides of the optimal value. The shape of the pH curve is reminiscent more of an enzymatic process than of an ionic exchange mechanism. The tertiary amine Pk 3010*aa* also shows a similar pH curve.

At a pH higher than 9·2 the disruption again increases, probably due to the alkalisation of the milieu, since the control values show a similar increased spontaneous disruption when the pH surpasses this value.

The disruptive action of decylamine, on the other hand, shows no pH peak but increases continuously with rising pH. This observation is consistent with the assumption that decylamine might act according to the displacement theory. The more alkaline the medium, the less ionised does decylamine become. Its lipid solubility increases and thereby its ability to penetrate the mast cell membranes.

The histamine-releasing action of 48/80 on intact tissue is described as "explosive," most of the histamine being released in the first few minutes after administration of the compound. From isolated mast cell granules, on the other hand, 48/80 releases histamine somewhat slowly, the amounts of histamine released increasing linearly with time. These observations, too, seem to be inconsistent with a displacement theory. If this theory were correct, the speed of the histamine release ought to be as high from the granules as from the cells.

Theory of Högberg and Uvnäs

The aim of the discussion above was to show that even though some experimental evidence can be found to support each of the various theories advanced, all of them seem to have serious shortcomings. In my view, an acceptable theory has to be consonant with at least two apparently incompatible observations, namely:

(1) The liberation of histamine is due to an enzymatic mechanism; and

(2) the histamine is stored in the mast cell granules in weak (ionic) linkages which do not require enzymatic processes for their dissolution.

As will be seen from the experiments to be described, it might be possible to combine the two observations in one theory.

Högberg, Thufvesson and Uvnäs[20] isolated highly active histamine-liberating fractions from *Ascaris lumbricoides* (from swine) and *Cyanea capillata* (jellyfish). These compounds as well as compound 48/80 disrupted mast cells of rat mesentery *in vitro* and released histamine from the perfused cat paw. Both processes were blocked by a polysaccharide fraction isolated from hip seeds[21]. Polyanions of high molecular weight reversibly inhibit different enzymes[22]—an inhibition believed to be due to blocking of free amino groups of the enzymes.

Attempts were therefore made to find experimental support for the hypothesis that 48/80 disrupts mast cells by activating an enzymatic mechanism[18]. Mast cells from rat mesentery were incubated with 48/80 in the presence of various metal ions and other enzyme inhibitors. The action of 48/80 was inhibited by some heavy metal ions, Zn^{++} ions, some enzyme inhibitors such as phenylhydrazine, iodoacetic acid and γ-tocopherylphosphate, and some high molecular weight polyanions such as polyphloretin phosphate, polyestradiolphosphate and polysaccharides. Although suggestive of an enzymatic mechanism, the observations did not warrant any conclusion about the more intimate nature of a possible enzyme.

The mast cells were incubated with about thirty various enzymes. Only one, lecithinase A, prepared from bee venom and from various haemolytic snake venoms, had the ability to disrupt mast cells. The lytic action on the mast cells was blocked by many of the inhibitors which blocked the effect of 48/80. Of special interest was the finding that trypsin and fibrinolysin were among those enzymes which, even in very high concentrations, were unable to disrupt the mast cells (see Table I).

The action of 48/80 is temperature dependent. The disruptive action decreases with falling temperature and disappears at temperatures below 10°. This blocking effect is reversible, the disruptive action being restored if the incubation fluid is reheated to 37°. Heating the mast cells above 45 to 50° also abolished the action of 48/80. This blocking was irreversible, however, the disruptive action of 48/80 failing to reappear on cooling of the incubation fluid to 37°. Such heated mast cells were still vulnerable to the lytic action of lecithinase A prepared from bee venom or to snake venoms as reported by Högberg and Uvnäs[16].

The following theory was propounded to explain our results (Fig. 3). A lytic enzyme is situated at the mast cell surface. The enzyme is normally inactive, since the active group is blocked by an inhibitor I. When

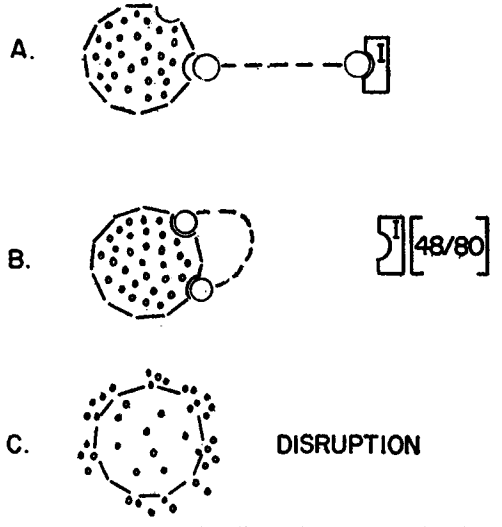


FIG. 3. Enzyme theory to explain the disruptive action of 48/80 on rat mesentery mast cells (for explanation see above).

this is removed by the liberator (48/80 in Fig. 3) the enzyme becomes active and attacks the cell membrane.

Recently Rathlev and Rosenberg[23] described a method for the phosphorylation of amines in aqueous neutral solution with a new compound, 1:3-diphosphoimidazole (DPI). Especially primary aliphatic amines seem to be easily phosphorylated with DPI. Högberg (1957, unpublished observations) reported that DPI inactivated enzymes with essential amino groups such as hyaluronidase and lecithinase A. DPI was found to cause an instantaneous inactivation of these enzymes.

It was observed by Högberg and Uvnäs that on simultaneous incubation of mast cells with DPI and the tertiary amine Pk 3010*aa*, no disruption of the mast cells occurred. In fact the mast cells become resistant to subsequent exposure to 48/80.

The reason for using compound 3010*aa* instead of 48/80 when treating the cells with DPI was that 48/80 is a secondary amine, i.e., has the group $N\begin{matrix}CH_3\\H\end{matrix}$ and thus could have been phosphorylated by DPI to $N\begin{matrix}CH_3\\P\end{matrix}$— and thereby possibly loose its liberating properties. Pk 3010*aa* is the corresponding tertiary amine $\left(N\begin{matrix}CH_3\\CH_3\end{matrix}\right)$ of 48/80 and cannot therefore be phosphorylated.

The blocking action of DPI might be explained as follows (see Figure 4). Pk 3010*aa* removes the inhibitor I from the NH_2 group of the lytic mast

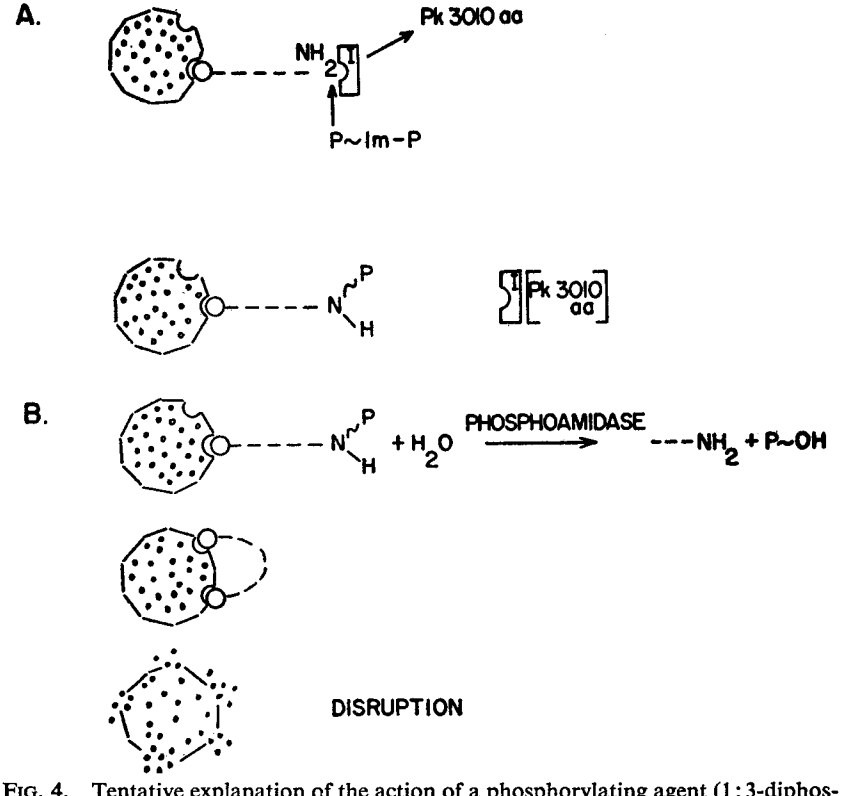


FIG. 4. Tentative explanation of the action of a phosphorylating agent (1:3-diphospholmidazole) or the mast cell enzyme (for explanation see above).

cell enzyme. The enzyme is thereby activated and the mast cell would be disrupted if the NH_2 group were not immediately phosphorylated due to the presence of DPI.

A test of the assumption that the resistance of the DPI-treated mast

cells to 48/80 was due to phosphorylation of a membrane enzyme would be to dephosphorylate, and thereby reactivate, the enzyme. Indeed, incubation of the "phosphorylated" mast cells in phosphoamidase caused a disruption of the mast cells (Fig. 4). Phosphoamidase did not disrupt "normal" mast cells. It is also of interest to note that the DPI treated cell membrane remains sensitive to the action of lecithinase A added from the outside.

Acetylation and several other processes that are thought to block NH_2 groups also inhibited the action of 48/80.

Taken together, the experiments lend strong support to the hypothesis that 48/80 disrupts mast cells by activating a lytic enzyme on the cell surface. The term "disruption," as mentioned previously, must not be taken too literally, however, since it is not known whether the mast cell membrane really is demolished or if the degranulation merely implies transient permeability changes in the membrane.

Comments upon the Theory of Högberg and Unväs

Our theory obviously has at least one weak point. It can easily explain, I think, the passage of histamine from the cell sap through a more or less damaged cell membrane. But how are we to explain the passage of histamine out of the intracellular granules, which seem to have a membrane not freely permeable to histamine? Several hypotheses can be advanced, however.

The activation of a lytic enzyme on the mast cell surface presumably results in the splitting of the phospholipid membrane into fatty acids and lysolecithins. The lysolecithins are strongly lytic, and it is easy to envisage a lysis of the granular membranes when they come into contact with the lysolecithins. The subsequent changes in the intragranular chemical milieu will result in a release of the histamine from its loose ionic linkage.

Another possibility is that the intracellular granules containing histamine are attacked by the same enzyme which breaks down the mast cell membrane.

The discharge of intracellular granules from the mast cell has been ascribed to an energy-requiring process. Junquiera and Beiguelman[19] observed in phase contrast microscopy the rapid formation of vacuoles, swelling of the cells and extrusion of the granules when 48/80 was added to living mesentery spreads from rats. The degranulation was believed to be due to metabolic activity inside the cell since it could be blocked by SH-blocking compounds (*p*-Cl-Hg benzoate, *O*-iodosobenzoate, iodoacetate), by substances blocking phosphorylative processes (2:4-dinitrophenol, arsenite and urethane), and by uranil nitrate, a metabolic inhibitor described as acting on cell membrane enzymes.

In my opinion the concentrations of enzyme inhibitors used by Junqueira and Beiguelman are such that specific inhibitory actions cannot be expected.

Is it really necessary to postulate the involvement of an enzymatic process in the transport of intracellular granules to the cell surface? The

granules are lipid structures floating in the cell sap just as oil drops float in water, and as such they are presumably very sensitive to changes in surface tension. It is not difficult to conceive changes in the membrane permeability giving rise to disturbances in the lipid-water interface with displacement, and changes in form and size of the granules. The contact with the activated membrane enzyme and formed lysolecithins might accelerate the release of histamine from the granules floating in the cell sap.

Although it may be tempting to attribute the release of histamine by 48/80 merely to activation of a lytic enzyme, the possibility cannot yet be ruled out that 48/80 triggers not only one but several enzyme mechanisms such as Junqueira and Beiguelman[19] have suggested.

The question arises, of course, whether a lytic cell enzyme may be assumed to take part in the mast cell disruption and histamine release produced by agents other than 48/80. Our observations that polysaccharides block not only the effect of 48/80 but also the action of the histamine liberating fractions from ascaris and cyanea as well as the histamine liberation caused by antigen-antibody reactions in guinea pig tissue, might indicate at least one common link in the chain of events which eventually lead to histamine liberation under these circumstances. The common link might be the activation of a lytic mast cell enzyme.

One argument against a lypolysis theory has been that little or no haemolysis occurs in anaphylactic shock, peptone shock, intoxication with ascaris products, and so on. The absence of haemolysis is not, in my opinion, a valid objection to a lypolysis theory. If a lytic enzyme (phospholipase) is anchored to the mast cell surface, activation of the enzyme will not necessarily bring about a spread of active enzyme in the blood. An enzyme attached to the mast cell membrane or to membrane fragments is unlikely on any major scale to come into contact, in active form, with circulating erythrocytes. In fact, a lytic mast cell enzyme might be very specific and lack the ability to attack the red cell membrane.

As pointed out by Mongar[14], the activity of histamine releasers varies considerably according to the species, the tissue and the methods used for determining their activity. The discrepancy might be a thousandfold. For instance, the concentrations of 48/80 required for the release of histamine from perfused[24] or chopped[25] guinea pig tissue, or from isolated intracellular granules from dog liver[26], are rather high and of about the same magnitude as those required for corresponding histamine release by simple amines such as octylamine. Under such conditions the histamine releasing action of 48/80 might be ascribed rather to a direct lytic action on the extra- and intracellular membranes than to activation of specific enzymatic mechanism.

In this discussion I have not touched upon the histamine release observed to occur in blood, especially in the rabbit and guinea pig. Our knowledge of the histamine release from thrombocytes and other formed elements of the blood, and of the role and nature of anaphylatoxin, is still too scanty to allow any fruitful discussion.

Recent discoveries concerning the role of the mast cells as histamine

bearers, and the possibility of denuding them of their histamine, have invigorated the discussions on the physiological role of histamine. Almost all authors take it for granted that histamine has important biological functions, the main arguments, of course, being the well-known pharmacological actions on smooth muscle and glands. Yet do these arguments carry any real weight? It might be that histamine for some reason is formed and stored in the mast cell, but that physiologically it does not pass through the cell membrane to the surrounding tissue. The presence of mast cells in vessel walls has led to the hypothesis that the mast cell histamine plays a role in the regulation of vascular tone. However, so far as I am aware, there is no experimental support for such an hypothesis. The presence of mast cells around the vessels may indicate transport of histamine from or towards the blood stream.

I have no suggestion to offer about physiological role of histamine and I think it would be unduly bold to dispute the physiological significance of this pet of so many laboratories. But the fact that histamine may, under pathological and experimental conditions, be released from the mast cell does not necessarily mean, I would stress, that such a release reflects a physiological mechanism.

References

1. Feldberg, *Histamine*, Ciba Symp., London, 1956, p. 4.
2. Riley and West, *J. Physiol.*, 1953, **120**, 528.
3. Cass, Riley, West, Head and Stroud, *Nature, Lond.*, 1954, **174**, 318.
4. Schayer, *Amer. J. Physiol.*, 1956, **186**, 199.
5. Copenhaver, Nagler and Goth, *J. Pharmacol.*, 1953, **109**, 401.
6. Blaschko, *Histamine*, Ciba Symp., London, 1956, p. 381.
7. Rocha e Silva, *Arq. Inst. Biol. Sao Paulo*, 1938, **9**, 145.
8. McIntire, White and Sproull, *Arch. Biochem.*, 1950, **29**, 376.
9. MacIntosh and Paton, *J. Physiol.*, 1949, **109**, 190.
10. Paton, *Pharmacol. Rev.*, 1957, **9**, 269.
11. Feldberg and Talesnik, *J. Physiol.*, 1953, **120**, 550.
12. Ungar, *Lancet*, 1947, **1**, 708.
13. McIntire, *Histamine*, Ciba Symp., London, 1956, pp. 420, 443.
14. Mongar, *ibid.*, p. 444.
15. Feldberg and Kellaway, *J. Physiol.*, 1938, **94**, 187.
16. Högberg and Uvnäs, *Acta physiol. scand.*, 1957 (in press).
17. Mongar and Schild, *Nature, Lond.*, 1955, **176**, 163.
18. Mongar and Schild, *J. Physiol.*, 1956, **132**, 30P.
19. Junqueira and Beiguelman, *Texas Rep. Biol. Med.*, 1955, **13**, 69.
20. Högberg, Thufvesson and Uvnäs, *Acta phys. scand.*, 1956, **38**, 135.
21. Högberg, Südow, Thon and Uvnäs, *ibid.*, 1957, **38**, 265.
22. Diczfalusy, Fernö, Fex, Högberg, Linderot and Rosenberg, *Acta chem. scand.*, 1953, **7**, 913.
23. Rathlev and Rosenberg, *Arch. Biochem. Biophys.*, 1956, **65**, 319.
24. Feldberg and Mongar, *Brit. J. Pharmacol.*, 1954, **9**, 197.
25. Mongar and Schild, *J. Physiol.*, 1952, **118**, 461.
26. MacIntosh, *Histamine*, Ciba Symp., London, 1956, p. 20.