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EFFECT OF CHAIN LENGTH OF ALIPHATIC AMINES ON HISTAMINE POTENTIATION AND RELEASE

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Diamines in the series $NH_2.(CH_2)_n.NH_2$ specifically potentiate histamine contractions of the guinea-pig ileum and inhibit the enzymatic destruction of histamine. These activities are greatest with short-chain compounds ($n \simeq 5$). Diamines also release histamine from isolated tissues and depress the contractility of plain muscle and the motility of paramecia. These activities increase with chain-length and are probably limited only by solubility. The parallelism between histamine-releasing activity and toxicity also extends to the monoamines in the series $CH_3.(CH_2)_{n-1}.NH_2$. Histamine-releasing activity of both series increases with increase of *p*H and can mainly be attributed to the non-ionized base.

From the results on the effect of chain-length and ionization on activity, it is suggested that aliphatic amines release histamine by penetration of the cell membrane in the non-ionized form, followed by exchange in the ionic form with intracellular histamine.

Within the homologous series of monoamines $CH_3.(CH_2)_{n-1}.NH_2$ and diamines $NH_2.(CH_2)_n.NH_2$ are found compounds which are destroyed by diamine oxidase (Blaschko and Hawkins, 1950), inhibit the enzyme competitively (Zeller, 1942), potentiate the actions of histamine (Arunlakshana, Mongar, and Schild, 1954), and release histamine (MacIntosh and Paton, 1949).

This paper describes the influence of chain-length and *p*H on these properties. Part of the results have already been briefly described (Mongar and Schild, 1953a).

METHODS

Inhibition of histaminase was measured by incubating various concentrations of diamines with a crude histaminase preparation consisting of an acetone-dried powder of pig kidney cortex. Potentiation of histamine contractions of the guinea-pig ileum was estimated in terms of the equivalent histamine dose, taking the dose before adding the potentiator as unity. These methods have been described in detail (Arunlakshana *et al.*, 1954).

For the quantitative measurement of the depression of the motility of paramecia, the concentration of amine required to immobilize half the organisms in a given colony in 5 min. was determined. This was done by pipetting equal volumes of a suspension of *Paramecium aurelia* into six 2 ml. perspex cups fitted into the revolving stage of a low-powered microscope (Fig. 1). Each cup contained several hundred organisms. At zero time, a different concentration of amine was added to each cup and at intervals during the following 60 min. the colonies were repeatedly observed in turn by rotating the stage. Above a certain concentration all paramecia became immobilized within 1 min. Below 1/10 of this concentration the organisms were unaffected. With intermediate concentrations the fraction of paramecia still swimming about was estimated.

The concentrations of all amines when expressed on a w/v basis are given in terms of the chlorides.

RESULTS

Inhibition of Histaminase.—The activity of diamines of different chain lengths in inhibiting the destruction of histamine by histaminase is shown in Table I. Inhibition was measured by incubating histamine with histaminase in the presence of various concentrations of amines. The

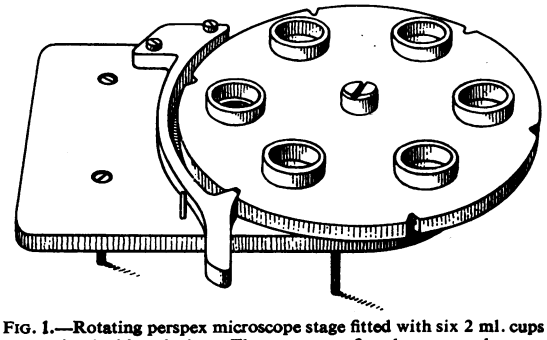


FIG. 1.—Rotating perspex microscope stage fitted with six 2 ml. cups and a locking device. The contents of each cup can be conveniently examined in quick succession.

TABLE I

INHIBITION OF HISTAMINASE BY STRAIGHT CHAIN ALIPHATIC DIAMINES

Histamine destruction by histaminase is inhibited by the presence of diamines. The table gives the reduction in the amount destroyed as % of that destroyed in controls containing no diamine. A different preparation of enzyme, in the form of acetone-dried pig kidney cortex, was used in each series of experiments. Histamine destruction in the controls was 60% in Series I and 80% in Series II.

	Chain Length	Concentration of Diamine				
		10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M.	10^{-2} M
Series I . .	C_5	Nil	11	4½	26½	49
	C_{10}	,,	Nil	6	18	10½
	C_{15}	4	,,	Nil	10	(insol.)
,, II	C_2	—	,,	4	7½	72
	C_4	—	,,	8	18½	86
	C_6	—	,,	2	—	40
	C_8	—	,,	1	10½	12

time of incubation was chosen to give 60 to 80% destruction of histamine in the absence of inhibitor. Table I gives % inhibition of destruction by various concentrations of diamines. As a group these compounds were weak inhibitors of histaminase, but their activity varied according to chain length. None produced an appreciable effect below a concentration of 1 mM. Concentrations producing 20% inhibition were determined, when necessary by extrapolation, from a plot of % inhibition against the logarithm of the concentration. Fig. 2 shows the pI_{20} values (Blaschko, Bülbring, and Chou, 1949). Inhibiting activity is maximal at about C_4 and falls off on either side. The C_{15} compound has only about 1/100th of the maximal activity.

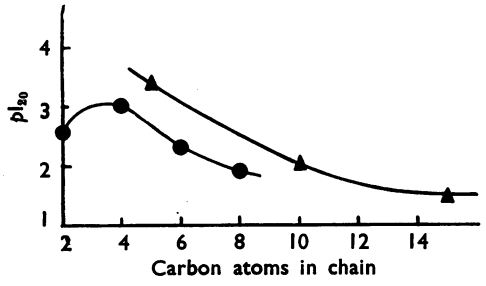


FIG. 2.—Activity of diamines as inhibitors of histaminase. The negative logarithm of the concentration required to produce 20% inhibition has been plotted against chain length for two series of experiments each with a different preparation of histaminase. Upper curve, series I diamines; lower curve, series II diamines (see Table I).

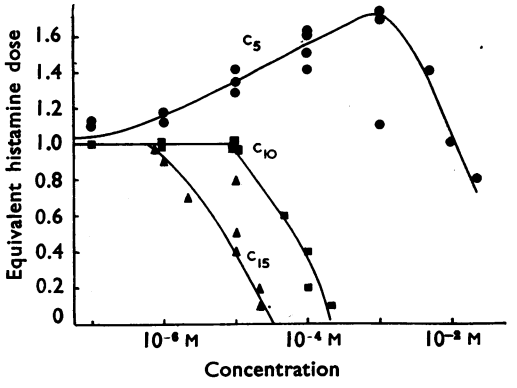


FIG. 4.—Potentiation and depression of histamine contractions of the guinea-pig ileum by various concentrations of diamines with chain lengths of 5, 10, and 15 carbon atoms.

Potentiation.—Fig. 3 shows the potentiation of the histamine contractions of guinea-pig ileum by cadaverine (pentamethylenediamine). When cadaverine was added to both washing and drug solutions it caused an immediate and persistent increase of the effect of histamine although it did not itself cause contraction of the ileum. Compounds of longer chain length, which produced only a weak inhibition of histaminase, did not potentiate but only antagonized histamine. Fig. 4

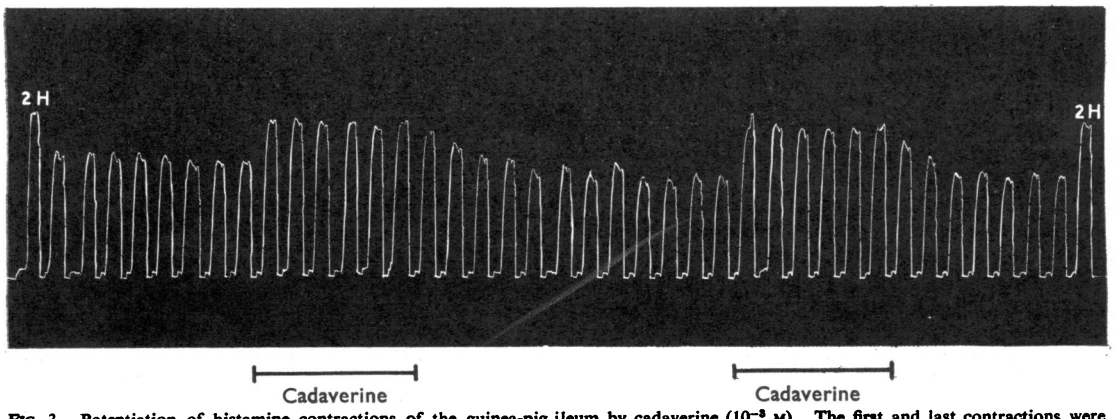


FIG. 3.—Potentiation of histamine contractions of the guinea-pig ileum by cadaverine (10^{-3} M). The first and last contractions were produced with a dose of 20 ng. histamine (2H) and the intermediate ones with 10 ng.

shows the effect of diamines with chain lengths of 5, 10, and 15 carbon atoms on histamine contractions of the guinea-pig ileum. The C_5 compound produced potentiation which increased with concentration up to 1 mM. and subsequently declined; high concentrations produce a depression. The C_{10} and C_{15} compounds produce only depression of histamine contractions. This depressant effect, which increased with chain length, is considered in more detail in a later section. The shapes of the curves in Fig. 4 can be explained in terms of the two opposing factors, potentiation and depression, both of which increase with concentration.

Histamine-releasing Activity.—The histamine-releasing activity of diamines has been demonstrated by MacIntosh and Paton (1949), by the test of the delayed fall in blood pressure in the cat anaesthetized with chloralose. The results reported have been obtained using minced guinea-pig lung (Mongar and Schild, 1953b), and isolated rat diaphragm (Rocha e Silva and Schild, 1949) to measure histamine-releasing activity of diamines of chain length C_5 to C_{15}. Fig. 5 shows the results

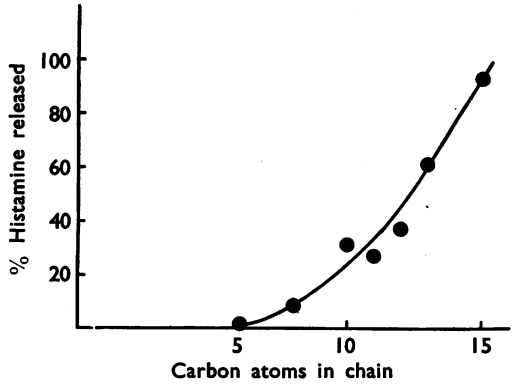


FIG. 5.—Histamine release from minced guinea-pig lung by 1 mM. solutions of straight-chain aliphatic diamines of various chain lengths. The amount released in 15 min. is expressed as % of the total tissue histamine.

obtained with equimolar solutions of diamines acting on minced guinea-pig lung. The activity increases steadily with chain length from less than 5% release with cadaverine to more than 90% release with pentadecamethylene-diamine during a test period of 15 min.

The effect of all diamines increased steeply with concentration. Fig. 6 shows that even cadaverine releases histamine in high concentrations. In a concentration of 1 mM. it is inactive both on minced guinea-pig lung and rat diaphragm, but at 100 mM. it releases about 80% of the histamine content of

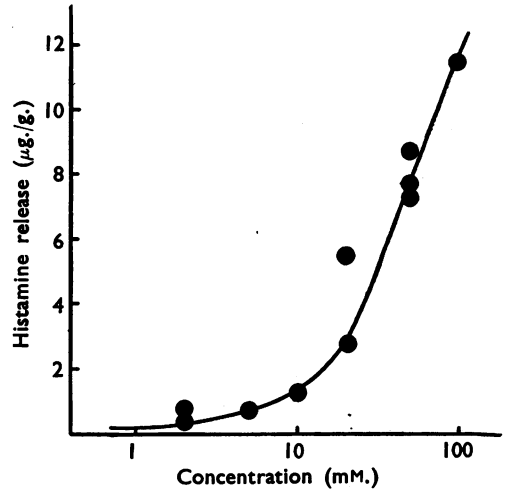


FIG. 6.—Histamine release in 10 min. from isolated rat diaphragm by various concentrations of cadaverine.

the tissue. The quantity of histamine released from minced guinea-pig lung in 60 min. by two concentrations of 5 different diamines is shown in Table II. From these results the concentrations of releaser giving 50% release in 60 min. were estimated, assuming a common regression coefficient. The values obtained are plotted against

TABLE II

PERCENTAGE OF HISTAMINE CONTENT OF GUINEA-PIG LUNG RELEASED IN 60 MIN. BY VARIOUS CONCENTRATIONS OF DIAMINES

Chain Length	Concentration of Diamine					
	$3{\cdot}2\times10^{-5}$	10^{-4}	$3{\cdot}2\times10^{-4}$	10^{-3}	$3{\cdot}2\times10^{-3}$	10^{-2}
C_5	—	—	—	—	45	75
C_8	—	—	45	64	—	—
C_{10}	—	—	53	102	—	—
C_{12}	—	56	68	—	—	—
C_{15}	31	64	—	—	—	—

chain length in Fig. 7*a*. In contrast to antihistaminase activity, histamine-releasing activity increases steadily with chain length.

Antihistamine Activity.—As shown in Fig. 4, high concentrations of diamines produce a depression of the histamine contractions of the guinea-pig ileum, and this depression increases with chain length. The antagonism for histamine has been determined quantitatively and the specificity of this antagonism has been investigated.

The antagonism measurements were done on the guinea-pig ileum; the antagonist was in contact for sufficient time to produce a steady depression. The concentrations of a number of diamines required to depress the effect of a double dose of histamine to that of a single dose were

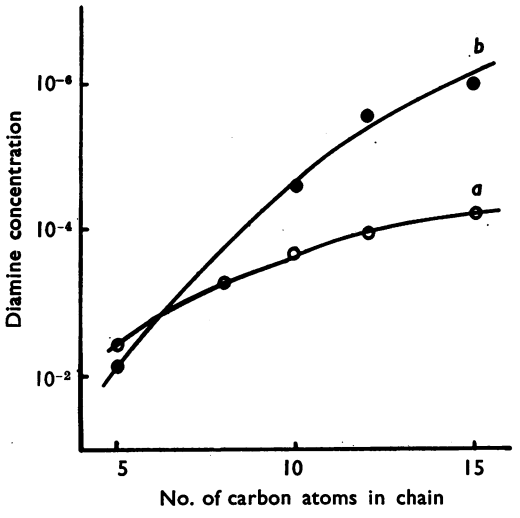
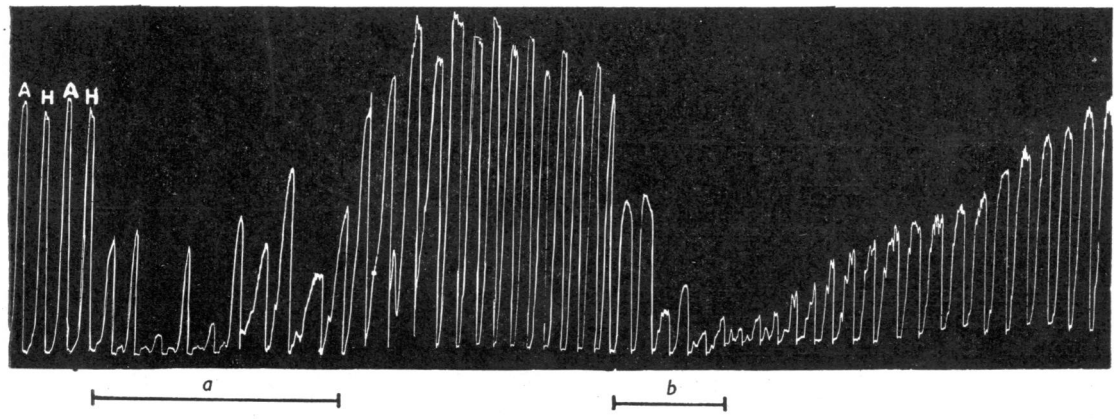


FIG. 7.—Effect of chain length on activity of diamines. *a*, Histamine release from guinea-pig lung: *b*, antagonism of histamine contractions of guinea-pig ileum.

determined (Fig. 7*b*). Antihistamine activity increases more steeply with chain length than the releasing activity; thus C_{15} is 7,000 times more active than C_5 as an antagonist but only 60 times more active as a releaser.

To determine whether this antagonism is specific, the antagonism towards acetylcholine was also measured. Experiments in which antagonism for histamine and acetylcholine was measured concurrently are illustrated in Fig. 8. Alternate equiactive doses of the two stimulant drugs were given. Following the addition of diamines C_{10} and C_{15} and the monoamine C_8 the effects of both stimulant drugs were depressed to roughly the same extent and after removal of the antagonist the contractions recovered at approximately the same rate. The responses to acetylcholine were slightly more reduced than those to histamine. These results suggest that the antagonistic effect of the amines is unspecific.

Toxicity to Paramecia.—Since the antagonistic effect of the long-chain amines was suggestive of



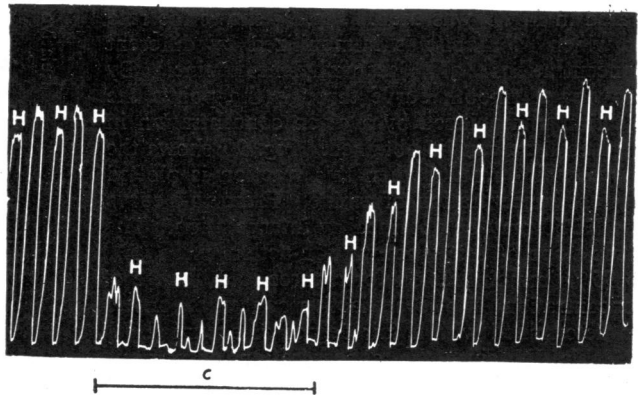


FIG. 8.—Depression by monoamines and diamines of contractions of guinea-pig ileum produced by alternative equiactive doses of histamine and acetylcholine. *a*, 2×10^{-5} $NH_2.(CH_2)_{10}.NH_2$; *b*, 10^{-6} $NH_2.(CH_2)_{15}.NH_2$, and *c*, 10^{-5} $CH_3.(CH_2)_7.NH_2$, were present in the bath fluid.

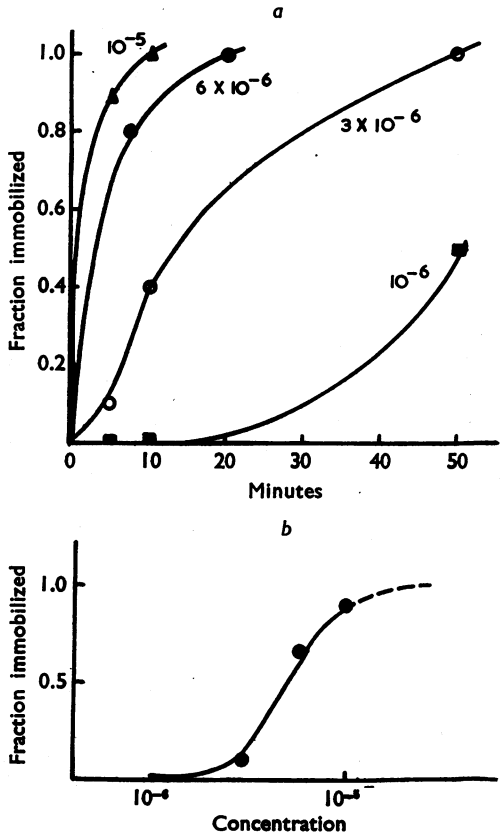


FIG. 9.—Characteristics of the paramecia toxicity test. *a*, Time action curves with various concentrations of decamethylene diamine. *b*, Concentration-action curve for a time of 5 min.

an unspecific depression of cell function rather than a specific antagonism, the toxicity of these compounds to unicellular organisms was also studied. A convenient measure was their activity in depressing the motility of paramecia. In order to obtain quantitative results the procedure illustrated in Fig. 9 was used. Fig. 9*a* shows the rate at which *P. aurelia* is immobilized by different concentrations of decamethylenediamine. By drawing a smooth curve through the points the fraction immobilized by a given concentration in a given time can be obtained. Fig. 9*b* shows the curve which results when this fraction is plotted against concentration of amine. From this curve the ED50 may be obtained by interpolation. The steep slope of the concentration-action curve (5% to 95% immobilization for a three-fold change of concentration) allows equiactive concentrations to be determined with a fair degree of accuracy.

Table III gives the concentration of various amines required to immobilize 50% of the organisms in 5 min. The activity of diamines increases steadily with chain length. The toxicity for paramecia of the monoamines also depends on chain length, but has a maximum at C_{12}.

TABLE III

TOXICITY OF AMINES TO PARAMECIA

The approximate concentrations required to immobilize 50% of the organisms in 5 min. are given. All concentrations are $\times 10^{-5}$

Monoamines		Diamines	
Chain Length	Conc.	Chain Length	Conc.
C_4	400	C_6	300
C_6	300	C_8	200
C_8	20	C_{10}	60
C_{10}	2	C_{12}	20
C_{12}	0·6	C_{14}	10
C_{14}	2·1	C_{16}	1

Comparison of Histamine-releasing Activity and Toxicity to Paramecia.—Fig. 10 shows the toxicity to paramecia of mono- and di-amines and also

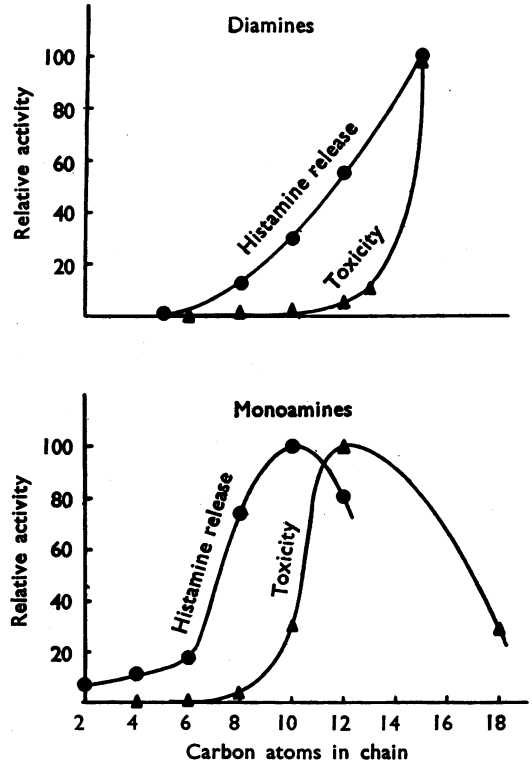


FIG. 10.—Effect of chain length on histamine-releasing activity and toxicity to paramecia for two series of aliphatic amines. The concentrations of the most active members of the series were: Monoamines: histamine release C_{10}, 7.2×10^{-5}; toxicity C_{12}, 0.6×10^{-5}. Diamines: histamine release 6.0×10^{-5}; toxicity 1.0×10^{-5}. The histamine-releasing activity is expressed in terms of the most active member of the series studied. The figures for histamine release by monoamines are from Mongar and Schild (1953b).

their histamine-releasing activity expressed in terms of the most active member of each series. The curves for histamine-release and toxicity are similar. Those for the diamines show a steady increase of activity with chain length with no suggestion of a maximum having been reached with the C_{15} compound. Those for the monoamines show a maximum by both tests which is probably due to micelle formation. Micelle formation does not occur with monoamines of 8 or less carbon atoms, but is known to occur with longer chains when a critical concentration is exceeded (Ralston and Hoerr, 1942). The critical concentration decreases with increase of chain length. The explanation of the maximum being due to micelle formation is borne out by the finding that the maximum of the curve for toxicity occurs at a greater chain length than the maximum for histamine release. Since lower concentrations are required to immobilize paramecia than to release histamine, the critical micelle concentration is reached earlier in the latter test.

Effect of p*H on Histamine-releasing Activity.*— Monoamines and diamines are both strong bases with pK_a of about 10.8 (Hoerr, McCorkle, and Ralston, 1943), and at *p*H 7 they are almost completely ionized. If the histamine-releasing activity of these solutions were due to the cation, change in *p*H would be expected to have little effect on activity. If on the other hand the histamine-releasing activity were due to the minute amount of non-ionized base present, an increase in *p*H would produce a marked increase in its concentration and hence would be expected to increase histamine release.

Fig. 11 shows the effect of *p*H on the histamine-releasing activity of octylamine and decamethylenediamine. The compounds are inactive at *p*H 7, but their activity rises sharply with *p*H to reach a very high value at *p*H 8.5. The question arises whether the increased histamine-release in alkaline solution is due to a change in ionization of the histamine-releaser or to an action on the tissue itself. The following findings suggest that the tissue itself is not involved: (i) There is no appreciable histamine release in Tyrode solution between *p*H 6.5 and 8.5. (ii) When a histamine-releaser is used whose ionization is not affected by *p*H, such as the quaternary compound dodecyltrimethylammonium chloride, histamine-releasing activity does not increase appreciably with *p*H. These results suggest that the striking effect of *p*H on the activity of the primary amines is due to a change in ionization of the releaser.

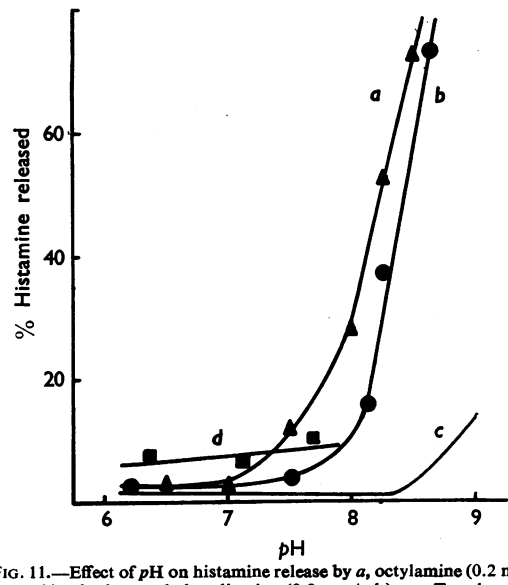


FIG. 11.—Effect of *p*H on histamine release by *a*, octylamine (0.2 mg./ml.); *b*, decamethylenediamine (0.2 mg./ml.); *c*, Tyrode solution; *d*, dodecyltrimethylammonium chloride (0.2 mg./ml.).

In a concentration of 0.2 mg./ml., the activity of octylamine and decamethylenediamine resides completely in the non-ionized form, since the histamine-releasing activity disappeared completely when the *p*H was reduced. If histamine-releasing activity were due solely to undissociated base, the *p*H-release curves obtained with different concen-

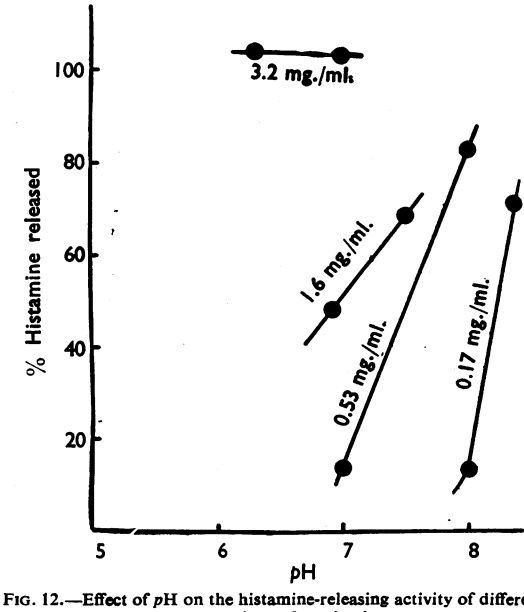


FIG. 12.—Effect of *p*H on the histamine-releasing activity of different concentrations of octylamine.

trations of the same releaser would be parallel. However, as shown in Fig. 12, this is not so. The curves obtained with different concentrations of octylamine are not parallel but become progressively less steep with increasing concentration of releaser. This means that as the concentration is increased the ionized molecules play an increasing part in histamine release and the contribution of the non-ionized molecules decreases. Fig. 13*a* shows the concentration of octylamine required to produce the same effect (50% histamine release) at different *p*H. From these results, and the pK_a of octylamine, the concentration of free base present at various *p*H's was calculated and is shown in Fig 13*b*. If the histamine-releasing activity resided entirely in the free base this curve would be horizontal. When the *p*H is high and the concentration of releaser is low the curve approaches the horizontal at a concentration of about 1 μg./ml. The free base thus appears to be highly active, producing a 50% histamine release at a concentration of 10^{-6}.

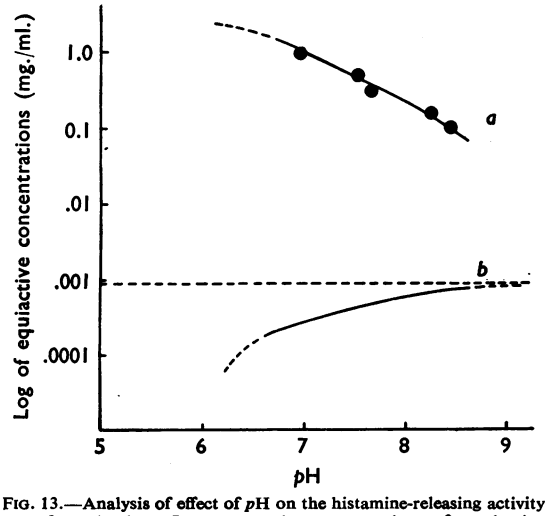


FIG. 13.—Analysis of effect of *p*H on the histamine-releasing activity of octylamine. In curve *a*, the concentrations of octylamine required to release 50% of the tissue histamine at 5 different *p*H have been plotted. In curve *b*, the corresponding concentrations of non-ionized base present at different *p*H have been calculated, based on a *p*K of 10.8.

When the *p*H is low and the concentration of releaser is high, the curve deviates from horizontal, indicating that the part played by the ionized base becomes increasingly important. This agrees with the finding that the fully ionized quaternary bases in high concentration are able to release histamine. Fig. 14 shows this for dodecyltrimethylammonium chloride (0.5 mg./ml.) and tubocurarine (1.7 mg./ml.). The activity of the quaternary compound

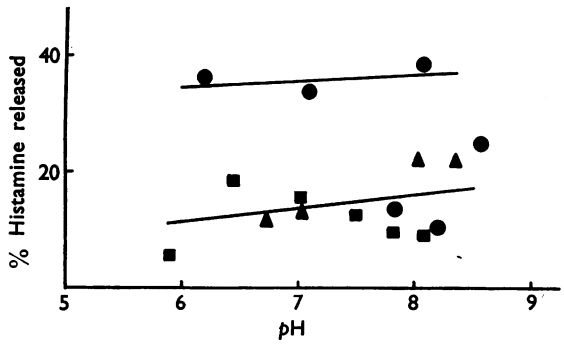


FIG. 14.—Effect of *p*H on histamine release from guinea-pig lung by quaternary amines. The release with C_{12} (0.5 mg./ml.) is depicted by the upper curve. The results with tubocurarine (1.7 mg./ml.), shown by the lower curve, were obtained from three separate experiments.

cannot be due to the presence of a small amount of the tertiary base, as the activity would not then be independent of *p*H. However, the most active quaternary compound is still 1,000 times less active as a histamine releaser from guinea-pig lung than the non-ionized octylamine molecule.

In investigating the effect of structure on histamine-releasing activity, a long-chain fatty acid (octanoic) and a weak base (aniline) were also studied. Both compounds were quite inactive when tested in relatively high concentrations (2 mg./ml. for octanoic and 1 mg./ml. for aniline).

DISCUSSION

The effects of diamines fall into two groups: one type of effect has a maximum with a chain length of 4 or 5 carbon atoms. The other type increases steadily with chain length until it is limited by insolubility or micelle formation.

The short-chain compounds have three apparently related properties: they are substrates for histaminase; they inhibit histaminase competitively; and they potentiate the effects of histamine. It has been shown by Zeller (1942) that diamines of less than 4 carbon atoms are not as readily destroyed by diamine oxidase as those with 4 and 5 carbon atoms. Blaschko and Hawkins (1950) have shown that compounds with more than 5 carbon atoms become increasingly resistant to diamine oxidase. Our results on the inhibition of histaminase by diamines also show a maximum activity at C_4 or C_5, suggesting that inhibition of the destruction of histamine is due to a competition for the active enzyme centres.

The potentiation of the effects of histamine by cadaverine can be explained on similar lines. It has previously been shown that many inhibitors

of histaminase, including cadaverine, selectively potentiate the pharmacological effects of histamine, and that their effectiveness is correlated with their antihistaminase activity (Arunlakshana *et al.*, 1954). This interpretation of the potentiating effect of cadaverine is further supported by the finding that the long-chain diamines C_{10} and C_{15}, which produce little or no inhibition of histaminase, also fail to potentiate histamine.

It could be argued that cadaverine potentiates histamine not by inhibiting histaminase but by releasing histamine. It is, however, unlikely that histamine release can account for the potentiation, because the concentrations of cadaverine needed for histamine release are 1,000 times greater than those which potentiate histamine. It would also be expected that potentiation by histamine release would be non-specific. On the contrary, potentiation by cadaverine is specific and only occurs with compounds which are destroyed by histaminase (Arunlakshana *et al.*, 1954).

Each of the three effects of the short-chain compounds can thus be explained in terms of a specific fit of enzyme receptors requiring a critical distance between the polar groups on the molecule. The remaining three effects, histamine release, antagonism of histamine, and toxicity to paramecia, are also related in a common way to chain length: they occur only with the long-chain compounds and increase with chain length. The fact that there is a maximum with the monoamines is almost certainly due to the formation of micelles. An increase of activity with chain length is frequently found with compounds which exert a non-specific narcotic or toxic effect on cells, and can be explained by a decrease in solubility in the aqueous phase and a simultaneous increase in solubility in the lipoid layer of the cell. Non-ionized molecules are much more active than ionized molecules, as shown in Table IV, which gives the effective concentrations of the *active moiety* of various histamine releasers. They include unpublished results for pentamethonium and Schild's (1949) data for ammonia. The table shows the effect of ionization and chain length on histamine-releasing activity. Ammonia itself in the non-ionized form is a relatively weak releaser acting in a concentration of nearly 1 mg./ml. In the monoamine series the addition of a 4-carbon chain increases the activity 200-fold, and the addition of an 8-carbon chain 1,000-fold. Similarly, for the diamines, the 5-carbon chain is 50 times, and the 10-carbon chain 1,000 times, more active than ammonia. Both types of compound lose practically all their activity if ionized: all the quaternaries tested, including tubocurarine, have a very low activity, less than 1/1,000th of that of the non-ionized bases.

TABLE IV

COMPARISON OF INTRINSIC ACTIVITIES OF HISTAMINE RELEASERS

Compound	Carbon Atoms in Chain	Equi-active Total Conc. mg./ml.	pK_a	Active Moiety	Conc. of Active Moiety at *p*H 8·2 mg./ml.	Relative Activity
Primary mono-amines: Ammonia	—	8·0	9·26	Non-ionized base	0·67	1
Butylamine	4	1·4	10·8	,,	0·0035	200
Octylamine	8	0·2	10·8	,,	0·0005	1,000
Primary diamines: Cadaverine	5	5·0	10·8	,,	0·013	50
Decamethyl-enediamine	10	0·2	10·8	,,	0·0005	1,000
Quaternary amines: Dodecatri-methylam-monium	12	0·6	—	Ion	0·6	1·1
Pentameth-onium (di-quaternary)	5	12·0	—	,,	12·0	0·06

These results show that highly active aliphatic releasers possess the following properties: (i) They are basic molecules; for example, the monocarboxylic acid with an 8-carbon chain is completely inactive. (ii) They have a long hydrocarbon chain; ammonia is only weakly active. (iii) High activity is found mainly in the non-ionized form; the completely ionized quaternary compounds are only weakly active.

It is concluded from these results, obtained from simple but highly active molecules, that histamine release consists of two stages. The first is a non-specific uptake into the lipoid layer of the cell which involves the non-ionized form of the base; this conclusion is supported by the finding that the histamine releasers also have a non-specific depressant or narcotic action on cells as different as unicellular organisms and mammalian tissue cells. Once the base has entered the cell, the second stage of the release process takes place. This can be pictured as an ion exchange with histamine, since at the *p*H inside the cell both the releaser molecule and histamine are almost completely ionized.

In terms of this mechanism of action octylamine and decamethylene diamine are powerful histamine releasers, because (i) the non-ionized long-chain molecule can readily get into the cell and (ii) they are sufficiently strong bases to change back into the ionic form inside the cell. A very weak base such as aniline does not release hist-

amine, since although it fulfils the first requirement of lipoid solubility it is not sufficiently ionized to act as an ion exchanger inside the cell. The strong quaternary bases, on the other hand, fulfil the second requirement: they can act as ion-exchangers but they cannot readily get into the cell.

McIntire, Roth, and Sproull (1951) have proposed a different mechanism for histamine release by aliphatic monoamines from rabbits' blood. They found that the C_{18} primary amine released histamine and that its action could be antagonized by the C_{18} quaternary amine, which itself was inactive. This antagonism formed the basis for a "receptor" theory of histamine release with the primary and quaternary amines competing for the receptor sites. The findings can be interpreted as readily in terms of the proposed mechanism of non-specific uptake followed by ion exchange within the cell. The quaternary amine molecules are taken up by the cell by virtue of the lipoid solubility of their hydrocarbon chains, but, being fully ionized, are left with their "insoluble" charged heads still in the aqueous phase. While thus immobilized they would be able to antagonize histamine release by repelling the ionized releaser molecules.

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