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QUANTITATIVE MEASUREMENT OF THE HISTAMINE-RELEASING ACTIVITY OF A SERIES OF MONO-ALKYL-AMINES USING MINCED GUINEA-PIG LUNG

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A wide variety of compounds have been reported to release histamine from tissues, but there is no common basis for comparing their activity. They have been tested on different tissues and by different methods: in some investigations (MacIntosh and Paton, 1949 ; Collier and Macauley, 1952) an effect of the released histamine, such as skin wealing, or delayed blood-pressure response, has been used to indicate histamine release, in others the released histamine has been directly measured by pharmacological or chemical methods (Alam, Anrep, Barsoum, Talaat, and Wieninger, 1939 ; Rocha e Silva and Schild, 1949 ; Feldberg and Paton, 1951 ; McIntire, Roth, and Sproull, 1951). For the purpose of a quantitative comparison of histamine-releasing activity it is desirable that not only the amount of histamine released but also the concentration of the releaser at the site of action should be known. In previous experiments in this laboratory (Mongar and Schild, 1952) a method was used in which pieces of isolated tissue were immersed in a known concentration of releaser, and the amount of histamine diffusing out in a given time was measured. A disadvantage of this method is that, owing to the thickness of the tissues, the releaser fails to attain diffusion equilibrium inside. Thus, although the concentration of the releaser at the outside is known, its concentration in the interior is undetermined. In the present investigation this disadvantage has been largely overcome by the use of minced tissues.

Tissues are minced into small particles, suspended in Tyrode solution, and stirred. Despite the stresses involved in mincing, the particles maintain their histamine content and show a very low and constant rate of spontaneous histamine loss. When an active substance is added to this material it releases histamine in a reproducible way and at a rate that can be readily measured. We have investigated the effect of concentration and of time on histamine release under these conditions and have used as a measure of activity the concentration of releaser producing a given percentage depletion of histamine in a given time.

As an example of the use of this method the activity of a homologous series of straight-chained mono-amines, $C_nH_{2n+1}NH_2.HCl$, has been compared with that of a well-established releaser. The C_8 to C_{12} compounds of this series were found to be considerably more active in releasing histamine from guinea-pig lungs than compound 48/80 (Baltzly, Buck, de Beer and Webb, 1949) which had been previously shown by Feldberg and Paton (1951) to be the most active releaser known.

METHODS

Guinea-pig lung tissue was disintegrated by a small Latapie mincer (volume about 7 ml.) shown in Fig. 1. The tissue was forced through small holes in a perforated steel disc by advancing the plunger. At the same time rotating blades cut off the extruded material into small pieces. A comparatively coarsely minced tissue was obtained by using a disc with 1.5 mm. diameter holes and blades rotating so as to cut off pieces of 0.9 mm. length. A more finely minced tissue could be obtained from the coarse mince by using a disc with holes of 0.5 mm. diameter and cutting off at an average length of 0.4 mm. In practice, the pieces were not uniform and contained strands of connective issue. The particles of the coarse mince had an average weight of about 2 mg. and the fine material of about 0.25 mg. Most of the connective tissue (10% by weight of the original material) did not pass through the perforated disc. The minced tissue was washed on a coarse sintered glass filter for 10 to 15 minutes. This treatment resulted in a loss of weight of about 25%. The washing solution remaining in the minced tissue was removed by gentle suction, leaving a material which could be divided into a number of uniform samples for histamine release.

The material was divided into 12 uniform samples by means of the device shown in Fig. 1, consisting of a perspex disc 1.2 cm. thick with 12 holes of 7 mm.

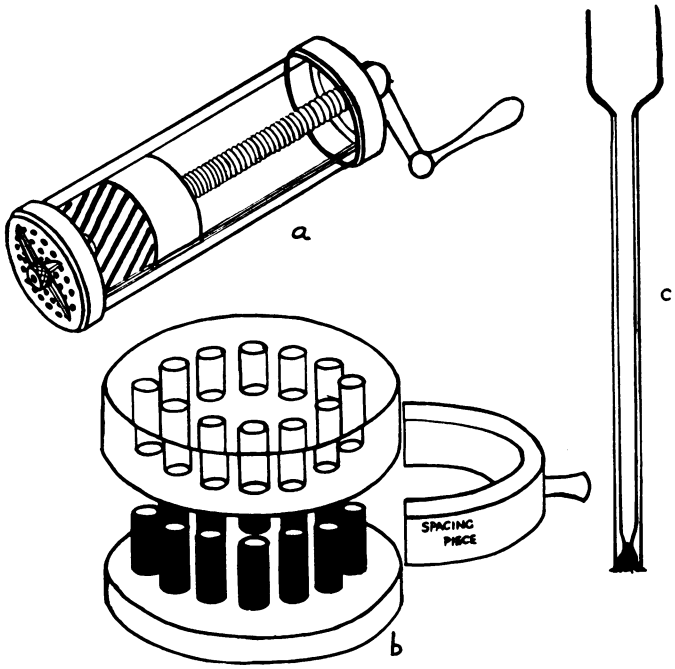

FIG. 1.—Apparatus used in connection with histamine release from minced tissues: (*a*) Mincer producing tissue particles of 2 mg. average weight. (*b*) Perspex sampler for producing twelve uniform samples of equal volume, 0.05 to 0.2 ml., according to the thickness of spacing pieces. (*c*) Filter tip of pipette for withdrawing liquid from a suspension of tissue particles. The filter pad consists of teased cotton-wool.

diameter. A second disc with 12 plungers fitting into the holes was used to close the bottom of the holes at a known depth as determined by the spacing pieces. The holes were then filled with the minced tissue level with the surface of the top disc. In this way 12 samples of identical volume and composition were obtained which could be extruded by removing the spacing pieces and pressing the two discs together.

The cylinders of minced tissue were transferred with a spatula to 10 ml. beakers containing 2 ml. Tyrode solution. The beakers were immersed in a bath at 37° C. and rocked from side to side through an angle of 60 degrees every second. An 8-mm. glass ball rolling across the bottom of each beaker gave efficient stirring without splashing particles of tissue on to the sides of the beaker above the level of the liquid. After 15 to 30 minutes at 37° C. the Tyrode washing solutions were removed and immediately replaced by 1-ml. portions of warm Tyrode solution containing different concentrations of histamine releasers, except for one beaker which was used as a control to determine the spontaneous rate of release of histamine.

To remove solutions from the minced tissue in the beaker a 5-ml. pipette with filter tip was used, consisting of a small cup plugged loosely with about 1 mg. cotton-wool as shown in Fig. 1. The end of the pipette was placed firmly on the bottom of the beaker, care being taken not to trap particles of tissue under it. Gentle suction then drew off most of the liquid without allowing the filter to get clogged. The remaining liquid adhering to the minced tissue was removed by gently stirring the contents of the beaker with the end of the pipette, keeping the tip pressed on the bottom, and sucking to remove the liquid so expressed. Finally the top of the pipette was closed and the filter pad along with any particles of tissue adhering to it returned to the beaker. This method of stirring and removing the fluid was evolved after a certain amount of experimenting. For example, an attempt was made to have a sintered glass filter permanently fitted in the bottom of a funnel-shaped beaker, and remove the test solution through it. This was not found practicable, since the particles tended to clog the filter. Neither was it possible to effect stirring by bubbling oxygen through the sintered glass filter because of excessive frothing.

The solutions containing the released histamine were boiled to stop bacterial action, and assayed on the guinea-pig ileum using a four-point method and automatic apparatus (Mongar and Schild, 1950). The histamine remaining in the minced tissue at the end of the experiment was released by boiling for three minutes in Tyrode solution, and similarly assayed. Control tests showed that grinding with sand was not necessary. Although the mono-amines produce some depression of the histamine response of the guinea-pig ileum, this was not a marked feature of the present experiments owing to the small volume of release solution used (1 ml. solution to 0.2 ml. tissue) which could be diluted as much as 100 times before assay. When depression occurred it was controlled by the addition of an equal concentration of mono-amine to the histamine standard. Control tests with atropine and mepyramine indicated that the activity of the test solutions was due to histamine.

Methylamine and ethylamine (B.D.H.) were used as the hydrochlorides. The higher amines, *n*-propylamine to *n*-decylamine, were supplied by Light & Co., and *n*-dodecylamine to *n*-octodecylamine by Armour & Co. (redistilled quality) as bases from which the hydrochlorides were prepared. 48/80 was obtained through the kindness of Dr. Kellaway, of the Wellcome Foundation.

RESULTS

Histamine in Minced Tissues

The histamine content of minced lung particles was found to be similar to that of whole lung. There was a loss of histamine on washing the coarse minced tissue with Tyrode solution, but there was a corresponding loss of weight of the tissue due to disintegration products passing through the filter so that the histamine content per unit weight of tissue remained the same. Similarly, there was no marked reduction of histamine content due to putting the coarse particles through the fine mincer. The results of a typical experiment are given in Table I, which shows the histamine content of (*a*) coarse lung particles immediately after mincing, (*b*) the same material after washing for 15 minutes with a large volume of Tyrode solution, and (*c*) the material after it has been put through the fine mincer and again washed. Although the weight of tissue is reduced to one-half, the histamine per gramme of tissue is reduced by only 16%. Histamine loss is apparently only from disrupted cells, the contents of which are mostly removed along with the histamine.

TABLE I

EFFECT OF MINCING ON HISTAMINE CONTENT OF GUINEA-PIG LUNG TISSUE

	Wt. of Tissue (g.)	Histamine Content (μg./g.)
(*a*) Coarse lung particles immediately after mincing	3·5	19
(*b*) The same material after washing for 15 minutes ..	2·5	19
(*c*) The material after fine mincing and washing ..	1·8	16

It was of interest to find out whether further mechanical disintegration of the cells would release all the histamine in the tissue as had previously been found for dogs' liver by Trethewie (1938). The finely minced tissue was treated in a Potter blender and the concentration of free histamine in the suspension was measured at various times during treatment. Fig. 2 shows that all the histamine could be released by this mechanical method, since no further release was obtained when the suspension was heated in a boiling-water bath for three minutes.

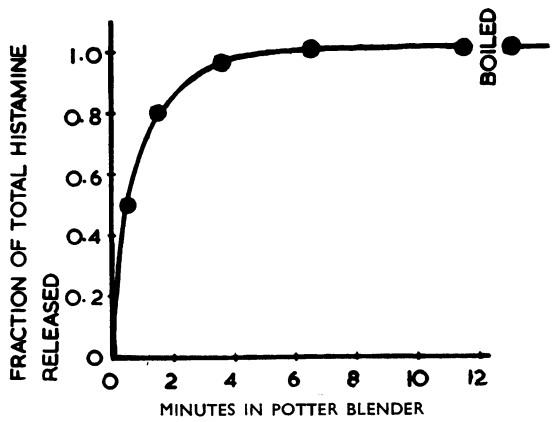

FIG. 2.—Complete release of histamine from minced lung tissue by mechanical disintegration (Potter blender and sand). Samples of the suspension were removed at intervals and immediately assayed.

Histamine Release from Minced Tissues

Spontaneous Release.—The rate of loss of histamine from minced unwashed tissue when suspended in successive lots of Tyrode solution was measured. The spontaneous rate of loss is high for the first 20 minutes, amounting to about 20% of the total histamine content. After 30 minutes the spontaneous rate of loss is small and almost constant at about 0.1%/min. (Fig. 3).

Action of Chemical Releasers.—Fig. 4 shows the shape of a typical release curve from minced tissue using compound 48/80 at a concentration of 10^{-3}.

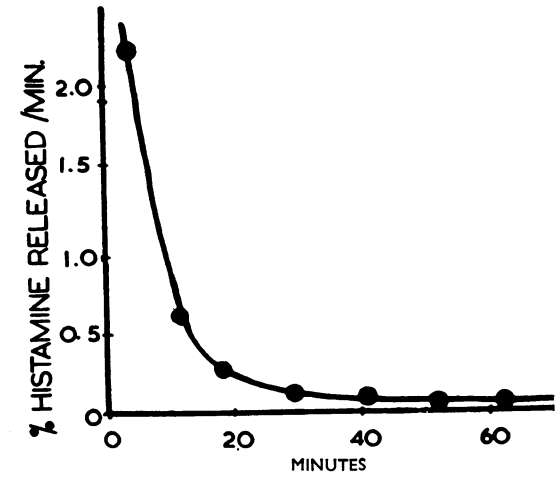

FIG. 3.—Rate of spontaneous release of histamine from minced guinea-pig lung tissue.

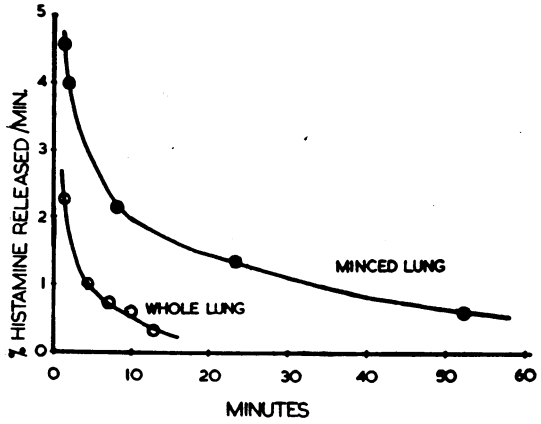

FIG. 4.—Rate of release of histamine by 10^{-3} 48/80 from minced and whole guinea-pig lung tissue.

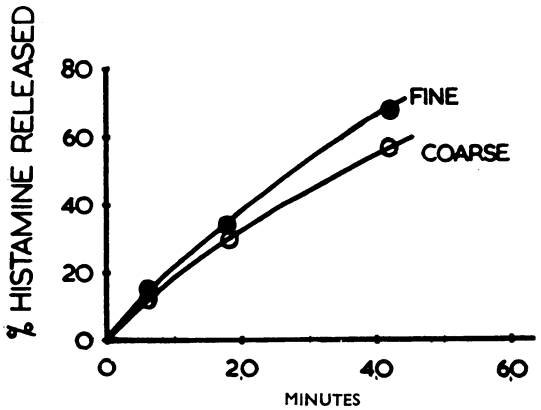

FIG. 5.—Effect of particle size on histamine release by 10^{-3} 48/80 (upper curve, average particle weight: 0·25 mg. Lower curve, average particle weight: 2 mg.)

Although the initial rate of release after adding 48/80 is over 50 times the spontaneous rate, the liberation of histamine is by no means instantaneous; it takes up to an hour for the release to be substantially completed. The rate of release from pieces of whole lung weighing about 100 mg. is shown for comparison (Mongar and Schild, 1952). It will be seen that release from minced tissue is much greater than for an equal weight of whole lung and continues to be measurable for a longer time.

Comparison of Coarse and Fine Mince.—Fig. 5 shows the time course of release from coarsely and finely minced tissue. Although the average particle weight of fine mince is only about one-eighth that of coarse mince, the rate of histamine release is not much greater. These results indicate that diffusion through tissue spaces does not determine the rate of release in minced tissues, hence little is gained by reducing particle size further by fine mincing. When whole tissues are used less histamine is released due to incomplete diffusion of the releaser, whereby an effective concentration is only attained in the superficial layers of the tissue.

Effect of Stirring.—Fig. 6 shows the effect of stirring by (1) rocking the beaker from side to side, and (2) increasing agitation by means of a glass

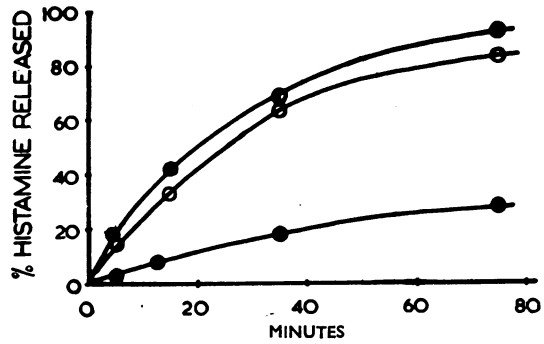

FIG. 6.—Effect of stirring on histamine release by 10^{-3} 48/80. ● = No stirring (bottom curve). O = Rocking beaker (middle curve). ◉ = Rocking beaker and glass ball (top curve).

ball rolling about on the bottom of the rocking beaker. These effects are compared with the rate of release obtained when the particles of tissue were suspended in Tyrode without stirring; thorough stirring is necessary to obtain maximal and reproducible rates of release.

Reproducibility.—Using the methods of subdivision, sampling, and stirring described, six replicate experiments gave satisfactory reproducibility as shown in Table II. A method is thus provided by which twelve or more experiments

TABLE II

UNIFORMITY OF HISTAMINE CONTENT AND REPRODUCIBILITY OF HISTAMINE RELEASE IN 6 PARALLEL SAMPLES OF MINCED GUINEA-PIG LUNG

Sample:	A	B	C	D	E	F
Total histamine in 0·2 ml. sample (μg.) ..	2·9	2·6	2·4	2·5	2·8	2·7
% spontaneous release in 10 min.	1·0	0·9	1·1	1·0	0·9	0·9
% release by 10^{-3} 48/80 in 10 min.	35	33	39	29	32	30

can be performed on material which is uniform both with regard to histamine content and histamine release.

Effect of Time and Concentration.—The rate of release of histamine by different concentrations of (*a*) 48/80 and (*b*) octylamine is shown in Fig. 7. All the histamine in the tissue can be released within 1 hour by 10^{-3} octylamine or 10^{-2} 48/80.

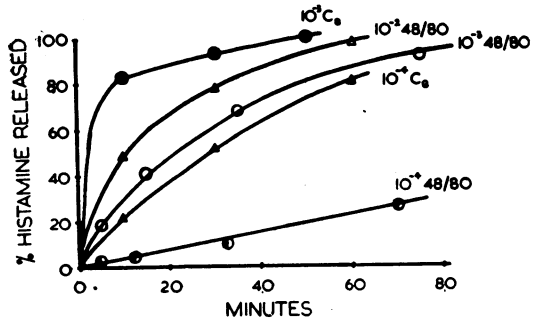

FIG. 7.—Time course of histamine released by different concentrations of 48/80 and of octylamine. Points on each curve were obtained from one sample of tissue by successive replacements of the releaser solution.

With lower concentrations the release is not complete in this time, but the shape of the curves suggests that they tend asymptotically to 100%. The curves are similar to those obtained with *d*-tubocurarine on the intact rat diaphragm (Rocha e Silva and Schild, 1949) except that in the latter preparation the maximal release was only 70% of the histamine in the diaphragm.

Comparison of Activity.—The concentration-release curves for a selected time can be used for comparing the activities of these compounds. Fig. 8 shows the curves for 10- and 60-minute release

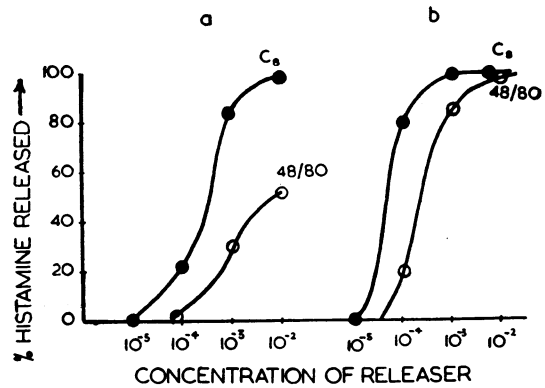

FIG. 8.—Concentration-release curves for octylamine and 48/80 with release times of (*a*) 10 and (*b*) 60 minutes.

times. It will be apparent that as the curves for 10 minutes are not parallel throughout their whole course the relative activities will depend to some extent on the release level chosen for the comparison. By using the longer time the shapes of the concentration-release curves become more nearly alike, and at the level of 25 or 50% release the slopes are sufficiently steep to make possible accurate comparisons. On the other hand, when very long periods for release are used the correction for spontaneous release becomes large: for weak releasers comparisons at the 10% level may have to suffice.

Activity of Mono-alkylamines

In view of the high activity of octylamine as a histamine releaser the series of straight-chained mono-amines, $C_nH_{2n+1}NH_2.HCl$, up to C_{18} was tested. Fig. 9*a* shows the concentration-release curves for release in 10 minutes and Fig. 9*b* curves for release in 60 minutes. With a release period of only 10 minutes, the weaker releasers cannot be given in sufficient concentration to produce a substantial effect, but with a period of 60 minutes they can be compared at the 50% release level. The concentrations giving this release are shown in

TABLE III

ACTIVITY OF AMINES $C_nH_{2n+1}NH_2.HCl$ AND 48/80 AS HISTAMINE RELEASERS FROM MINCED GUINEA-PIG LUNG. COLUMN (*a*) GIVES THE CONCENTRATIONS REQUIRED TO PRODUCE A 50% RELEASE IN 60 MIN.; COLUMN (*b*) THE ACTIVITIES RELATIVE TO THE C_{10} MEMBER OF THE SERIES

Compound	(*a*)	(*b*)
C_2	$1{\cdot}0\times10^{-3}$	7
C_4	$6{\cdot}3\times10^{-4}$	11
C_6	$4{\cdot}1\times10^{-4}$	18
C_8	$9{\cdot}8\times10^{-5}$	74
C_{10}	$7{\cdot}2\times10^{-5}$	100
48/80	$4{\cdot}7\times10^{-4}$	15

Table III. C_{10} is 14 times more active than C_2 and nearly seven times more active than 48/80.

Fig. 10 shows the amount of histamine released in ten minutes by equimolar concentrations of mono-amines of chain length C_1 to C_{14}; C_{10} is the most active compound. Reducing the chain length to six carbon atoms or increasing it to 14 greatly reduces or abolishes the releasing activity. Compounds of chain length greater than C_{14} cannot be tested at a concentration of 5×10^{-4} M owing to their low solubility; but a saturated solution of C_{18} (about 10^{-4} M) was inactive. This contrasts with the results of McIntire *et al.* (1951), who found that C_{18} was highly active in releasing histamine from rabbits' blood, whereas C_{12} was almost inactive.

DISCUSSION

It has been shown that minced guinea-pig lung provides suitable material for testing the activity of histamine releasers. A number of uniform samples may be prepared from this material for testing the activity of different drugs or different concentrations of the same drug. All the histamine present in the tissue can be released using this method, in contrast to previous diffusion methods using intact tissues in which only a fraction of the

histamine was liberated. The essential feature of this type of experiment is that the particles are large enough for most of the cells to remain intact and so retain their histamine, yet small enough to allow rapid diffusion of the releaser to all the cells. Since the rate of histamine release from minced tissue varies according to the concentration of releaser, and the concentration-action curves are generally steep and parallel, quantitative comparisons of activity are possible.

Tested in this way, the straight-chained mono-amines octylamine to dodecylamine are powerful histamine releasers considerably more active than 48/80. The maximum activity of the mono-amine with 10 carbon atoms is reminiscent of the finding of MacIntosh and Paton (1949) that in a series of diamines maximum histamine-releasing activity occurs with a similar chain length. Since many of the active histamine releasers previously tested, such as *d*-tubocurarine, compound 48/80, the diamidines, and the diguanidines, also have at least two basic groups separated by a large hydrocarbon moiety, it seemed reasonable to assume that their activity was due to the goodness of fit of two basic groups for bifunctional receptors in the tissue. Such an explanation is clearly inapplicable to the mono-amines which have only one basic group attached to the hydrocarbon chain, the length of which alters the hydrophobic nature of the molecule rather than any goodness of fit. Each carbon atom is capable of free rotation, so there will be a whole series of configurations for the chain, unlike that for a molecule with two polar groups of equal sign at the extremities which will tend to maintain it in a fully extended configuration. The maximum activity of decylamine may be the resultant of two opposing processes: increasing activity with increasing chain length opposed by an increasing tendency to form micelles with increasing chain length. The aggregation of molecules in solution would reduce the effective concentration of releaser. This explanation for the effect of chain length of activity is supported qualitatively by conductivity measurements (Ralston and Hoerr, 1942) which show that the C_{10} member of the series is the first one to show micelle formation.

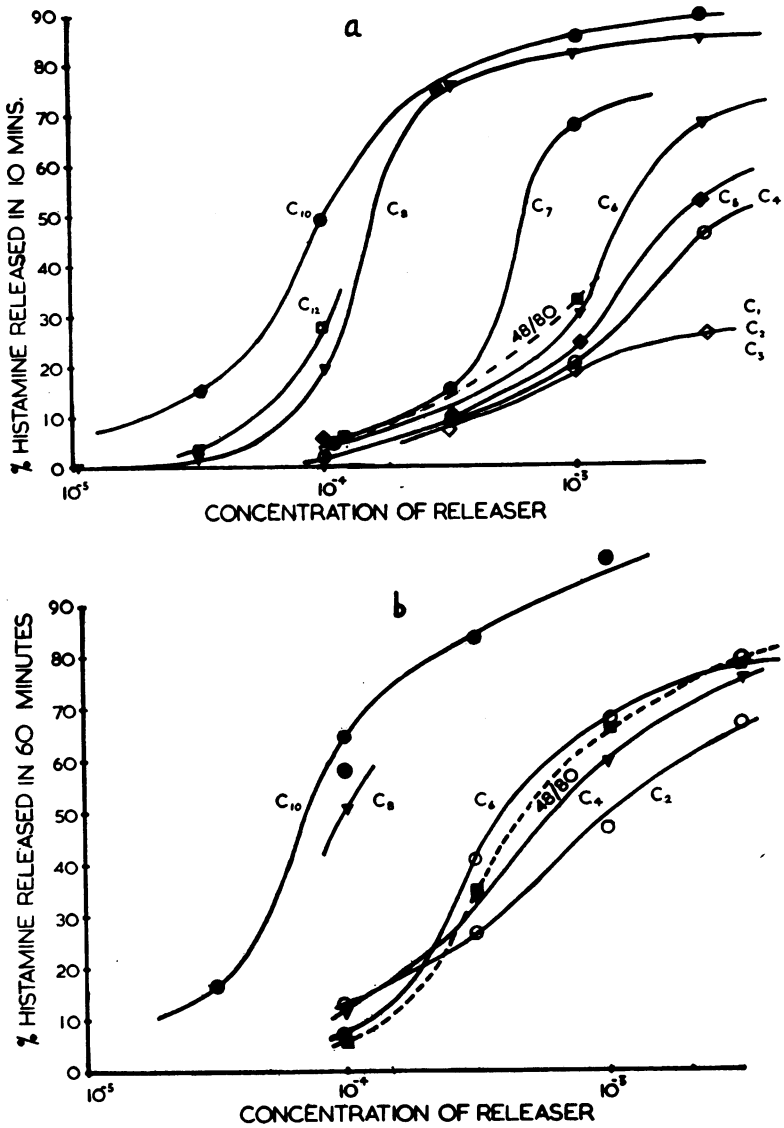

FIG. 9.—Concentration-release curves for a series of mono-amines with release times of (*a*) 10 minutes, (*b*) 60 minutes. The curves in (*a*) were obtained in separate tests from those in (*b*). Points in (*b*) are the results of single experiments, points in (*a*) represent the mean of several experiments.

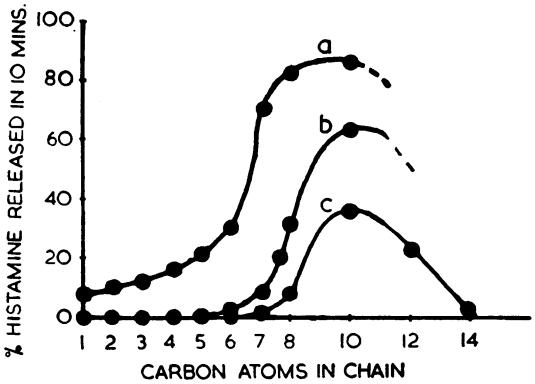

FIG. 10.—Effect of chain length on histamine release from minced guinea-pig lung by equimolar solutions of mono-amines $C_nH_{2n+1}NH_2.HCl$: (*a*) 10^{-2}M, (*b*) 10^{-3}M, (*c*) 5×10^{-4}M.

In unpublished experiments we have found that the mono-amines produce a triple response in human skin in concentrations similar to those needed for histamine release from minced guinea-pig lung. The effect of chain length on activity was also similar. On the other hand, the concentration of 48/80 required to produce wealing (about 10^{-7}) was very much less than that required for release from isolated tissues (about 10^{-4}), so that on the basis of skin tests 48/80 appears much more active as a histamine releaser. It cannot be stated at present whether these discrepancies are due to a fundamental difference between the two methods of assessing histamine-releasing activity (the one direct, the other indirect) or to tissue or species specificity.

SUMMARY

1. A method is described for obtaining particles of guinea-pig lung of 0.25 or 2 mg. average weight, and of dividing the material into a number of samples of equal histamine content.

2. This material has a histamine content similar to that of whole lung, and when suspended in Tyrode solution it shows a very low spontaneous rate of loss of histamine. When a histamine releaser is added, it releases all its histamine into the surrounding fluid, the rate of release depending on the concentration of releaser.

3. The concentration-release curves for a given time of contact are generally steep and parallel, and are therefore suitable for quantitative comparisons of the activity of different histamine releasers. The concentration of releaser needed to liberate half the histamine in 60 minutes has been chosen as a convenient index of activity.

4. The method has been used to measure the relative activity of the series of mono-amines $C_nH_{2n+1}NH_2.HCl$ up to C_{18}, and of compound 48/80. Decylamine was the most active member of the series. It was 14 times as active as ethylamine and nearly seven times as active as compound 48/80.

We wish to thank Miss Betty Harris for technical assistance.

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