Archives of Environmental Health: An International Journal
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/vzeh20

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Published online: 02 May 2013.

To cite this article: Margaret Hitchcock PhD , Dolores M. Piscitelli & Arend Bouhuys MD (1973) Histamine Release From Human Lung by a Component of Cotton Bracts, Archives of Environmental Health: An International Journal, 26:4, 177-182, DOI: 10.1080/00039896.1973.10666252

To link to this article: http://dx.doi.org/10.1080/00039896.1973.10666252

Histamine Release From Human Lung by a Component of Cotton Bracts

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Tyrode-soluble extracts of cotton bracts, pericarps, and fibers, and compound 48/80 were tested for their capacity to release histamine in vitro from chopped human autopsy lung. Average total histamine was 12.61 μg/gm (N = 25). Average histamine released nonspecifically (control), with bracts extract or with compound 48/80 was, respectively, 1.72 (N = 25), 0.47 (N = 8), 4.17 (N = 25) micrograms per gram of lung per 30 minutes. No release was obtained from extracts of pericarps or fibers. Bracts extract contained a steam volatile component that released an average of 1.20 (N = 9) micrograms histamine per gram of lung at 30 minutes. This component had similar physicochemical behavior to methyl piperonylate. Synthetic methyl piperonylate released histamine from chopped human lung when incubated at 5×10^{-11} to 5×10^{-5}M. The optimum concentration was 5×10^{-8}M when the average release of histamine was 1.35 (N = 4) micrograms per gram of lung per 30 minutes. Histamine release by methyl piperonylate was stimulated by propranolol hydrochloride and inhibited by isoproterenol hydrochloride and theophylline thus implicating a role for cyclic adenosine monophosphate in the release process.

It has been postulated that the acute reversible bronchoconstriction encountered following inhalation of cotton flax or hemp dust could be due in part to the local nonallergic release of histamine.[1] Bouhuys and Nicholls[2] have observed that inhalation of an aerosolized aqueous extract of the dust caused similar symptoms and responses of the lungs to those experienced following dust inhalation as determined by objective measurements of pulmonary function. Furthermore, similar extracts of cotton, flax, and hemp dusts released histamine in vitro from surgically resected chopped human lung.[3,4]

The purpose of this investigation was to determine the localization of the histamine releasing agent in the cotton dust and to identify its nature.

Methods

Drugs and Chemicals.—Tyrode buffer pH 7.4 was freshly prepared from stock solutions at the beginning of each experiment and contained the following (in micromols per milliliter): NaCl, 136.7; KCl, 2.6; $CaCl_2$, 1.8; $MgCl_2$, 0.49; $NaHCO_3$, 11.9; NaH_2PO_4, 0.29; and glucose, 5.55.

All drugs were dissolved in and diluted with Tyrode solution immediately before incubation. Deionized water was used throughout.

Analytical Procedures.—*Measurements of Fluorescence.*—A spectrophotofluorimeter was used and fluorescence spectra were determined at the maximum excitation wave length. A fluorescence intensity-concentration curve was obtained for methyl piperonylate in water at the maximum excitation wave length of 295 mμ and maximum emission wave length of 345 mμ.

Paper Chromatography.—Descending paper chromatography was carried out with Whatman Nos. 1, 4 and 3 mm paper, the solvent front being allowed to run approximately 16 inches from the starting point. Relevant reference compounds were run simultaneously with samples of the extracts undergoing examination. The solvents used were the following: butyl alcohol:acetic acid: water (4:1:5); butyl alcohol:ammonia:water (4:1:5); and isopropranol-ammonia (7:3).

Paper Ionophoresis.—This was carried out on horizontal Whatman No. 1 paper strips in a paper electrophoresis apparatus with a power supply with variable voltage and current. The current supplied was sufficient to maintain a constant potential differenc of 300 v for up to four hours.

Detection of Compounds on Paper Chromatograms and Ionophoretograms.—Piperonylic acid and methyl piperonylate were detected by exposure to ultraviolet light. They both quenched the fluorescence of paper at 270 mμ and gave a blue-purple fluorescence at 350 mμ.

Preparation of Plant Extracts.—Bracts, pericarps, and fibers were separated from the cotton plants. The extracts were prepared by grinding with a pestle and mortar, 6 gm of the appropriate plant part in 36 ml of Tyrode solution for 15 minutes. The resulting mixture was filtered through Whatman No. 1 paper and the filtrate was subsequently refiltered under suction through a Nalgene filter with a pore size of 0.45μ. One milliliter of the resulting filtrate was used per incubation in all of the experiments to be described.

Preparation of Lung Tissue.—Human autopsy lung obtained within 6 to 16 hours after death from patients whose primary cause of death was not due to lung disease was used in all experiments. All samples appeared grossly normal. The lung was

Submitted for publication June 19, 1972; accepted Sept 15.

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Read in part before the Federation of American Societies for Experimental Biology, Chicago, April 17, 1971.

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Table 1.—Histamine Characteristics of Human, Rat, and Guinea Pig Lungs

	μg/gm/30 min	Total Histamine, %
Human (N = 25)*		
Average total histamine	12.61 (3.34-29.35)	
Average control release	1.72 (0.48-3.2)	16.91(4.57-31.10)
Average net release by compound 48/80 (2 mg)	4.17 (0.65-10.80)	31.78 (11.89-55.44)
Average destruction of added histamine (3μg)	< 0.15 (< 5%)	
Rat (N = 8)†		
Average total histamine	6.87 (4.07-9.1)	
Average control release	0.34 (0.04-0.78)	6.48 (0.4-19.2)
Average net release by 48/80 (2 mg)	1.90 (1.36-3.25)	31.40 (16.6-47.1)
Average destruction of added histamine (3μg)	1.54 (51%)	
Guinea pig (N = 4)‡		
Average total histamine	31.59 (25.58-42.81)	
Average control release	0.28 (0.20-0.33)	0.89 (0.77-1.04)
Average net release by compound 48/80 (2 mg)	2.00 (1.08-4.16)	6.38 (3.83-13.11)
Average destruction of added histamine (3μg)	1.012 (34%)	

*Eleven men, average age 69; 14 women, average age 62.
†Refers to eight separate experiments. Each experiment took 12 to 20 lungs; the lower values for total histamine are from young rats.
‡Refers to four experiments. Each experiment took 6 to 12 lungs.

Table 2.—Histamine Released From Human Lung by Cotton Plant Extracts (N = 8)

	Av Histamine (μg/gm)
Control release/30 min	1.41 (0.48-3.57)
Release by compound 48/80/30 min	3.32 (1.88-5.82)†
Release by bracts extract/30 min	0.47 (0.30-0.63)†
Release by pericarps extract/30 min*	0.00†
Release by fibers extract/30 min*	0.00†
Total	**13.78 (6.75-24.00)**

*Data are from two experiments only.
†Data are corrected for control release.

Table 3.—Histamine Release From Human Lung by Bracts Distillate (N = 9)

	Av Histamine (μg/gm)
Control release/30 min	1.38 (0.78-2.80)
Release by compound 48/80/30 min	3.83 (1.31-8.49)*
Release by distillate/30 min	1.20 (0.38-3.40)*
Release by residue/30 min	0.00*
Total	**11.06 (5.63-18.64)**

*Data are corrected for control release.

kept in Tyrode buffer at pH 7.4 at all times. The tissue was dissected from the pleura, larger airways, and blood vessels, cut into approximately 8 cu mm pieces and chopped on a McIlwain tissue chopper two to four times. The chopped tissue was suspended in Tyrode solution, filtered under vacuum through a nylon stocking, and washed free of blood. All possible fluid was removed by suction. In all of the experiments to be described, 0.5 gm samples of the chopped lung tissue were weighed immediately prior to incubation.

The animals used in this study were adult female Hartley albino guinea pigs (approximately 300 gm) and adult male Sprague-Dawley rats (approximately 200 gm). They were killed by a blow on the head, exsanguinated, and the lungs removed and treated as described for human lung. Approximately 12 to 20 lungs were required for each experiment.

Incubation Procedure.—In all of the experiments to be described, weighed aliquots of chopped lung tissue were incubated in Tyrode buffer in a total volume of 5 ml. All incubations were conducted at 37 C for 30 minutes in a metabolic shaker with air as the gas phase. All incubations were performed in triplicate. Reagent blanks, tissue controls, and an internal histamine standard curve (0.2μg to 1.0μg histamine base) were also incubated simultaneously. In each experiment, the histamine released by compound 48/80 (2 mg) was also determined as a check that the lung tissue contained an adequate supply of intact mast cells. The amount of histamine destroyed by the amine oxidases and/or methylases present in the tissue was determined by the addition of 1μg to 5μg of histamine base to the lung tissue. Routinely 3μg histamine base were added. At the end of the incubation, the incubates were filtered through Whatman No. 1 filter paper and 2.5 ml of the filtrate was added to 0.5 ml of 30% trichloroacetic acid. The samples were refrigerated for at least 30 minutes for protein precipitation and then centrifuged at 1000 g for ten minutes. Supernatant, 2.5 ml, was assayed for histamine content by the spectrophotofluorimetric method of Shore et al.[5]

In all experiments the total histamine content of the lung was determined by grinding 200 mg of the chopped tissue in 0.5 ml 30% trichloroacetic acid. The mixture was diluted to 10 ml with Tyrode solution, filtered, and 2.5 ml of the filtrate added to 0.5 ml 30% trichloroacetic acid. The samples were assayed for histamine content as described. Total histamine content determinations were performed in quintuplet.

Results

Table 1 shows the average data collected from all of the human lung samples used in the series of experiments to be described. There was a great variability in the total histamine, probably due to inherent individual variation but also to the condition of the tissue at the time of the experiment and to variable loss of histamine during chopping and washing. The control (nonspecific) release of histamine and that released by compound 48/80 amounted to respectively 17% and 32% of the total. Table 1 does not include data from lung samples that had a nonspecific release greater than 35% of the total histamine. This proportion was taken as an indication that the lung was in poor condition at the time of the experiment, and data from such experiments were discarded. Under the experimental conditions described, negligible amounts of histamine were destroyed during incubation.

Data from rat and guinea pig chopped lung incubated under identical conditions to those used for human lungs are also given in Table 1. The data from these experiments reflect average values from 12 to 20 lungs. Thus, individual variations from lung to lung in a given species

could not be detected in animal experiments.

Histamine Released by Cotton Plant Extracts.—Table 2 shows the histamine released by Tyrode extracts of bracts, pericarps, and fibers. Only bracts extract released detectable amounts of histamine equivalent to an average of 3.4% of the total. Bracts extract released histamine in eight of 17 trials. All three extracts failed to release histamine when incubated with rat or guinea pig lung.

Purification of Bracts Extract.—It was observed that the histamine-releasing agent in the bracts extract was steam volatile and could be distilled from the extract under vacuum at about 40 C. Table 3 shows the histamine released from human lung by bracts distillate. No histamine was released by the residue that was redissolved in water to its original volume. The distillate had about three times the histamine releasing capacity as the original bracts extract and released an average of 10.8% of the total histamine. No attempt was made to adjust the salt concentration in the distillate, but in this series of experiments the control incubation media contained 4 ml Tyrode and 1 ml distilled Tyrode. Bracts distillate was a more consistent releaser than the original bracts extract and released detectable amounts of histamine in all trials with human lung. It failed to release histamine when incubated with rat and guinea pig lung.

Identification of Histamine Releaser in Bracts Distillate.—The physical and chemical properties of the histamine releasing agent in the distillate included the following:

1. It was steam volatile.
2. It absorbed ultraviolet light at 270 mμ and fluoresced at 350 mμ.
3. It failed to give the common color reactions on paper for amino acids, mucopolysaccharides, and reducing compounds.
4. It had a pleasant sweet odor.
5. It did not move from the origin in the chromatographic solvent systems tested.
6. It was destroyed during ionophoresis at pH 1.7 and 12.5.

Chu et al[6] described an unidentified ester of piperonylic acid which occurs in oak galls and which had some antihistamine activity at high concentrations. Since antihistamines are also under some conditions histamine releasers,[7] and since the methyl ester of piperonylic acid is steam volatile, the similarity of this compound with the histamine releaser in the bracts distillate was tested.

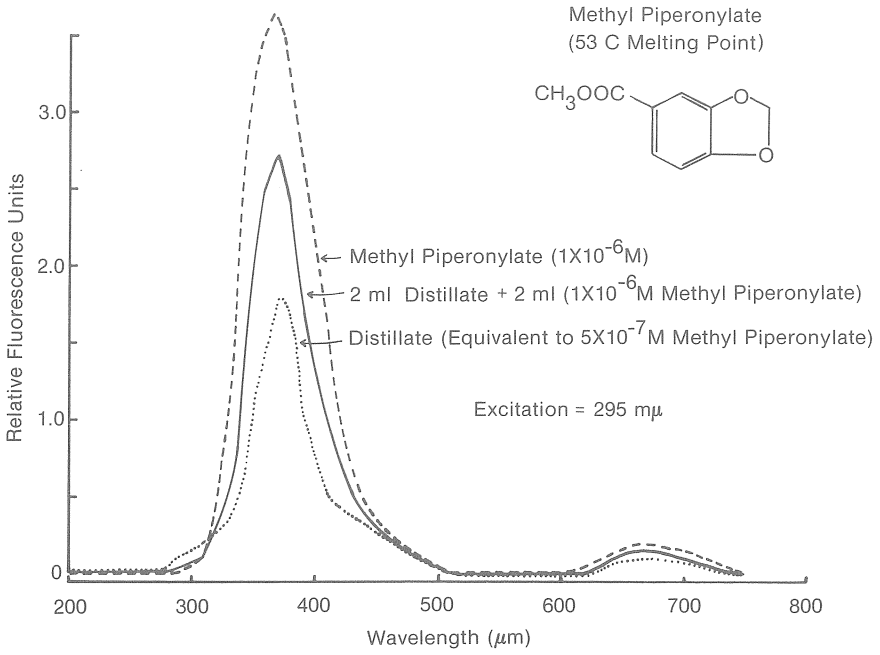

Fig 1.—Fluorescence spectra of methyl piperonylate and bracts distillate. The fluorescence intensity of distillate alone was equivalent to that produced by 5×10^{-7} M methyl piperonylate and was obtained by reference to a fluorescence intensity-concentration curve obtained for methyl piperonylate in water.

Methyl piperonylate was synthesized from the parent acid according to the method of Campbell and Taylor.[8] Figure 1 shows the fluorescence spectra of a 1×10^{-6}M solution of methyl piperonylate and of bracts distillate. All three solutions had identical fluorescence spectra. In addition they all had the same maximum excitation wavelength at 295 mμ. The synthetic methyl piperonylate was also destroyed by strong acid and alkali and failed to move in the solvent systems tested.

Histamine Release by Methyl Piperonylate.—Figure 2 shows the histamine released by various concentrations of methyl piperonylate. Detectable amounts of histamine were released when methyl piperonylate was incubated with chopped human lung at a concentration from 5×10^{-5} to 5×10^{-11} M. The average optimum concentration was 5×10^{-8} M although this varied from 5×10^{-6} to 5×10^{-10} according to the lung tested. No histamine was released when pooled rat and guinea pig lungs were incubated with similar concentrations of methyl piperonylate.

Data on the histamine releasing capacity of methyl piperonylate are shown in Table 4. The average amounts of histamine released by the distillate (Table 3) and methyl piperonylate were similar although individual releases from lung to lung by these two agents varied by an order of magnitude. The average histamine released by the distillate and by methyl piperonylate was nearly three times that released by the bracts extract (Table 2). From the fluorescence intensity of the distillate, it was calculated that an equivalent of approximately 10^{-7} M methyl piperonylate was present which would give a final molar concentration during incubations of the same order of magnitude was the average optimum concentration of methyl piperonylate.

Effect of Drugs on Histamine Release by Methyl Piperonylate.—The effects of propranolol hydrochloride, isoproterenol hydrochloride, and

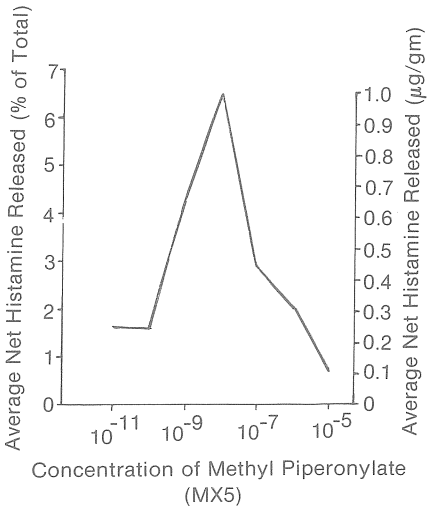

Fig 2.—Histamine release from human lung by different concentrations of methyl piperonylate. Data are the average obtained from five different lungs. Average total histamine was 15.28μg/gm. Data have been corrected for control (nonspecific) release which was 1.81μg/gm.

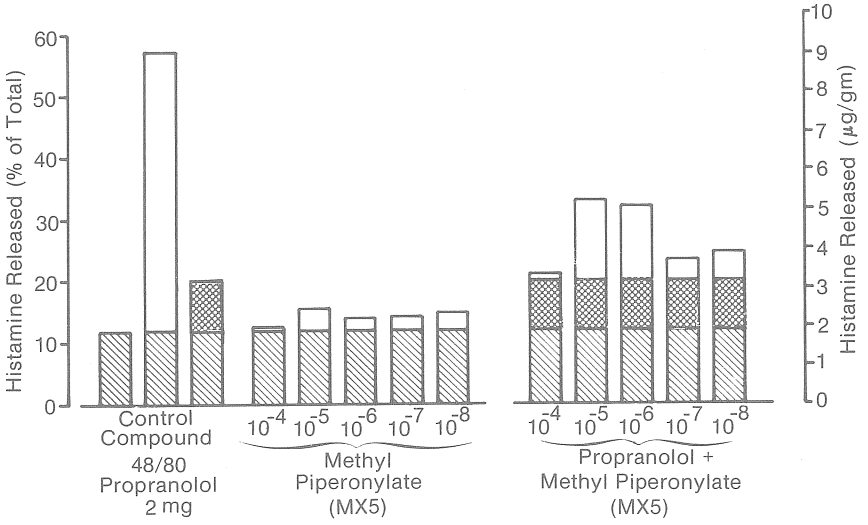

Fig 3.—Potentiation of histamine release from human lung by propranolol. Incubation conditions are described in "Methods." Propranolol was 1×10^{-4} M. Data are the average obtained from three separate lungs. Average total histamine was 15.83μg/gm. Shaded areas represent contribution to total histamine released by propranolol alone.

theophylline on the histamine released by methyl piperonylate were tested. Propranolol itself released histamine from chopped human lung when incubated at 1×10^{-2} to 1×10^{-5} M. Preliminary experiments established that propranolol concentrations of 1×10^{-4} M were the most useful in detecting an effect of this drug. Figure 3 shows the effect of propranolol on histamine release by 5×10^{-5} to 5×10^{-9} M methyl piperonylate. Propranolol increased the release of histamine at all concentrations of methyl piperonylate tested. Maximum increase occurred at 5×10^{-7} M methyl piperonylate when extra histamine equivalent to 15.5% of the total was released. Propranolol, at 1×10^{-4} M, also increased histamine released by compound 48/80 and caused some destruction of added histamine (Table 5).

Isoproterenol at 1×10^{-5} M inhibited the release of histamine by 5×10^{-6} to 5×10^{-8} M methyl piperonylate. Concentrations of isoproterenol lower than 1×10^{-5} M had no effect. Theophylline at 1×10^{-2} M also inhibited histamine released by methyl piperonylate but 1×10^{-3} M had no effect (Table 5).

Comment

Our experiments confirm the findings of Bouhuys and Lindell[3] and of Nicholls et al[4] that cotton dust contains a pharmacological agent that releases histamine from chopped human lung.

Surgically resected human lung has been extensively used as a model system for the study of histamine release in vitro,[3,4,9-14] but no intensive studies have been reported on the histamine-releasing properties of human autopsy lung. Information on the histamine content of human lungs is minimal, and the available data all come from resected lung. Schild et al[9] reported 55μg/gm in a young asthmatic, Nicholls et al[4] studied three lungs containing from 12.42μg/gm to 27.14μg/gm and Scheard and Blair[12] studied five lungs containing from 5.0μg/gm to 35.0μg/gm. The values reported in this study (Table 1) fall within the published range but tend to underestimate the amount originally present since some histamine was probably lost during preparation of the tissue.

Under the experimental conditions described, added histamine was not lost during the incubation procedure. This suggests that the histamine was not metabolized, destroyed, or taken up by the tissue, a finding that is in common with the unpublished observations of Jack L. Mongar, PhD (oral communication, February 1970). In contrast, Lilja et al[15] observed that 30% of added carbon 14 labeled histamine (2μg) was converted to methyl histamine by surgically resected chopped human lung. However, the conditions of their experiment (2 gm tissue incubated at 37 C for three hours) were such that considerable tissue destruction may have occurred. The concomitant liberation of intracellular enzymes such as transmethylases could conceivably explain the discrepancies between the two sets of data. In our study, approximately 26% of added histamine was destroyed when the chopped lung was incubated with high concentrations (1×10^{-3} M) of propranolol (Table 5). McKeever et al[16] have observed that propranolol makes the membranes of rabbit lung macrophages more permeable to dye uptake. Since large quantities of histamine were released when human lung was incubated with 1×10^{-3} M propranolol, it is possible that intracellular metabolizing enzymes were released at the same time, thus causing the destruction of the added histamine. When rat or guinea pig lung was incubated under the same conditions as human lung, the loss of added histamine was respectively 51% and 34% (Table 1). Bennett[17] reported that chopped rat

lung destroyed histamine approximately four times faster than chopped guinea pig lung. Moreover, he observed that when lung homogenates were used instead of chopped tissue, the rate of destruction by rat lung was increased by 146%, and that for guinea pig lung was increased by 39%. The increase in the rate of destruction as a result of homogenization of the tissue suggests that there are intracellular amine oxidases and/or methylases that are inaccessible to added histamine in a chopped lung preparation. The data from our investigation are difficult to compare with those of Bennett's since he added about 60μg histamine base to 160 mg (dry weight) chopped lung.

The studies to determine the localization of the histamine-releasing agent in the cotton plant revealed that only the bracts extract contained significant activity. No histamine could be detected following incubation with extracts of pericarps or fibers. Detectable amounts of histamine were released by the bracts extract in eight of 17 trials. The individual variation in the amount of histamine released by compound 48/80 was also wide and varied from 11.9% to 55.4% of the total histamine present (Table 1). Bracts distillate was more consistently active than the original bracts extract and released detectable amounts of histamine in all trials. Again the amount of histamine released by the bracts distillate from different lungs varied by an order of magnitude (Table 3). The individual variability in the sensitivity of the mast cell to different agents is not surprising and can be expected to be wide. Moreover, bracts extract may well contain substances that could inhibit the histamine release from some lungs.

Piperonylic acid is a naturally occurring compound and occurs in paracoto bark. It is related structurally to other naturally occurring material such as saffrole, the major constituent of sassafras. The physicochemical characteristics of piperonylic acid and its derivatives are similar and depend on the parent compound. However, since the histamine-releasing agent in the bracts distillate is steam volatile and since methyl piperonylate is the only derivative of piperonylic acid with this property, it was decided to test the similarity of the two materials. The most impressive characteristics that they have in common are (1) steam volatility, (2) identical fluorescence spectra, and (3) ability to release histamine from chopped human lung. The shape of the methyl piperonylate dose response curve is similar to the dose response curves obtained with the human sensitized leukocyte-antigen system[18] and the adenosine-triphosphate-rat peritoneal mast cell system.[19] The degree to which methyl piperonylate released histamine varied considerably from lung to lung (Table 4). Moreover, the optimum concentration for histamine release also shifted from lung to lung. Lichtenstein and Osler[20] similarly observed that human leukocyte sensitivity to a given antigen varied over a 10,000-fold concentration range. The low concentrations of methyl piperonylate required to release histamine from human lung make it an attractive candidate for in vivo activity.

Methyl piperonylate is not the only naturally occurring substance that releases histamine. Read et al[21] isolated an unidentified histamine liberator from the tannin fraction of *Eucalyptus robusta* logs. Moreover, the roots of *Carissa carandas* contain a factor that releases histamine from guinea pig lung.[22] Simple nitrogenous compounds as well as volatile low molecular weight piperidines have been isolated from hemp,[23] but the histamine-releasing properties of these compounds have not been reported on.

The histamine-releasing component in the bracts distillate may not be the only one present in the cotton dust, although material that is highly insoluble in aqueous media is unlikely to act in vivo. Our study has not taken into consideration other endogenous agents that might be released by cotton dust and that are active on bronchial smooth muscle.

Histamine release by bracts extract, bracts distillate, and methyl piperonylate at 5×10^{-8} M appears to be specific for human lung since both guinea pig and rat lung, when incubated under identical conditions, failed to release histamine. These negative findings with animal lung tissue support the previous observations of Davenport and Paton[24] and Nicholls et al[4] who failed to detect histamine release from the lungs of

Table 4.—Histamine Release From Human Lung by Methyl Piperonylate (N = 4)

	Av Histamine (μg/gm)
Control release/30 min	1.84 (0.97-2.67)
Release by compound 48/80/30 min	6.00 (2.78-10.80)*
Release by methyl piperonylate/30 min (5×10^{-8} M)	1.35 (0.38-3.4)*
Total	**19.93 (15.33-29.35)**

*Data are corrected for control release.

Table 5.—Effect of Drugs on Histamine Release and Destruction

Drug Combinations	Histamine Released* (% of Control)	Histamine Destroyed, %
Theophylline (1×10^{-2} M) + methyl piperonylate (5×10^{-7} M)	63.60	
Theophylline (1×10^{-2} M) + methyl piperonylate (5×10^{-8} M)	82.35	
Theophylline (1×10^{-2} M) + methyl piperonylate (5×10^{-9} M)	24.43	
Theophylline (1×10^{-2} M) + compound 48/80 (2 mg)	48.47	
Theophylline (1×10^{-2} M) + histamine (3μg base)		0.00
Isoproterenol (1×10^{-5} M) + methyl piperonylate (5×10^{-6} M)	61.27	
Isoproterenol (1×10^{-5} M) + methyl piperonylate (5×10^{-8} M)	0.00	
Isoproterenol (1×10^{-5} M) + compound 48/80 (2 mg)	96.53	
Isoproterenol (1×10^{-5} M) + histamine (3μg base)		0.00
Propranolol (1×10^{-4} M) + compound 48/80 (0.5 mg)	211.7	
Propranolol (1×10^{-4} M) + histamine (3μg base)		26.1

*All values given are a percentage of the releases obtained by the agents alone in the absence of drugs. The data are the average from three experiments.

several animal species when incubated with aqueous extracts of cotton dust. In contrast our studies have shown that all lungs, both animal and human, released histamine when incubated with compound 48/80 (Table 1).

Propranolol-stimulated methyl piperonylate and compound 48/80 mediated histamine release, whereas isoproterenol inhibited it, thus implicating a β-adrenergic receptor mechanism and possibly a role for cyclic 3′5′ adenosine monophosphate (AMP) either directly or indirectly in the histamine release process. This is supported by the observation that theophylline also inhibited histamine release. Moreover, Capurro and Levi[25] found that dibutyryl 3′5′ cyclic AMP inhibited histamine release from passively sensitized guinea pig hearts. Furthermore, Orange et al[26] observed that intracellular cAMP in human lung was increased by the β-adrenergic agents epinephrine and isoproterenol. Other investigators[10,11,13,14] have reported that isoproterenol inhibits histamine release from chopped lung, both animal and human, but at concentrations far below those that were effective in our experiments. However, all of the other studies used actively or passively sensitized chopped lung which might explain the greater sensitivity of the histamine release process to isoproterenol. Single cell systems appear to be more resistant to the inhibiting action of isoproterenol although it has been shown in the passively sensitized human leukocyte system of Lichtenstein and Margolis[27] and Assem and Schild.[11] However, isoproterenol failed to influence compound 48/80 induced histamine release from rat peritoneal mast cells.[28]

This investigation was supported in part by Public Health Service research grant UI 00435.

Russell H. Pope, MD, Chief Pathologist, and his staff of Bridgeport Hospital made the autopsy material available to us. Joseph B. Cocke of the Southeastern Cotton Ginning Research Laboratory, Clemson, SC, arranged for the collection of cotton plants.

Ayerst Laboratories provided the propranolol hydrochloride.

Nonproprietary and Trade Names of Drug

Propranolol hydrochloride—*Inderal.*

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