

# Characterisation of textile dust extracts: I Histamine release in vitro

J S DOUGLAS AND PAMELA G DUNCAN

From the John B Pierce Foundation Laboratory, New Haven, Connecticut 06519, USA

**ABSTRACT** Cotton, flax, hemp, and cotton bracts extracts did not release histamine from mouse, rat, guinea pig, horse, cow, and monkey lung. Neither rat peritoneal mast cells nor mouse mastocytoma cells released histamine when incubated with textile dust extracts. Compound 48/80 caused a considerable release of histamine from all these tissues and the extracts released histamine from pig and human lung tissues. Whereas dusts that cause bronchospasm in man released histamine—for instance, cotton—those that are inactive, such as pericarps, did not. Histamine release was not quantitatively related to the concentration of extract used. Cotton bracts extract released histamine from pig lung tissue whereas in the same preparations methyl piperonylate was inactive. The active releasing agent was highly water soluble but could not be steam distilled from, nor extracted by, ether from bracts extract. These physicochemical properties are not characteristic of methyl piperonylate. There was no correlation between the induction of histidine decarboxylase and the histamine releasing capacity of the extracts. We conclude that pig lung is a useful qualitative assay tissue for the further characterisation of the histamine releasing agent(s) in textile dust extracts.

The release of histamine from lung tissue is believed to play an important part in the aetiology of byssinosis.<sup>1</sup> This theory is supported by the observations that aqueous extracts of textile dusts release histamine from lung tissue in vitro<sup>2,3</sup> and that, in vivo, the acute bronchoconstriction occurring as a consequence of exposure to textile dust is prevented by the prior administration of antihistamine.<sup>4</sup> Recent work has suggested that there is more than one histamine releasing agent in cotton dust and these substances appear to be chemically unrelated.<sup>5,6</sup> Others have proposed that methyl piperonylate is the histamine releasing agent in cotton dust<sup>7,8</sup> since this molecule and closely related structures are present in many plants. It has also been suggested that byssinosis is associated with an exposure to bacterial endotoxins frequently found in retted vegetable fibres.<sup>9</sup> These toxins may act by altering tissue sensitivity or by producing histamine, or both.<sup>10</sup>

This report describes the histamine releasing

Presented in part at the International Conference on Byssinosis, Alicante, Spain, 1968, and at the meeting of the American Thoracic Society/American Lung Association, Cincinnati, Ohio, 1974.

Received 5 October 1982  
Accepted 6 December 1982

capacity of aqueous extracts of textile dusts when incubated with lung tissues from a number of species, including man. Histamine release from rat peritoneal mast cells and mouse mastocytoma cells and the histidine decarboxylase inducing capacity of the textile dust extracts are also described.

## Methods

### PREPARATION OF PLANT EXTRACTS

#### *Simple dust extracts*

Cotton bracts, pericarps, cotton dust, hemp, or flax dusts (retted or unretted) were ground to a fine powder in a Waring blender. The powder was mixed with Tyrode's solution (1 g powder/6 ml Tyrode's solution) and the mixture was allowed to stand at room temperature for several hours with occasional mixing. The composition of the Tyrode's solution was (mM concentrations): NaCl, 139.2; KCl, 2.7; CaCl<sub>2</sub>, 1.8; MgCl<sub>2</sub>, 0.49; NaHCO<sub>3</sub>, 11.9; NaH<sub>2</sub>PO<sub>4</sub>, 0.4; glucose, 5.5; pH 7.4. The aqueous extract was clarified by squeezing the suspension through cheesecloth to remove coarse material. The liquid was then filtered under vacuum via a Whatman No 1 filter paper through a Büchner funnel. The aqueous extracts were subjected to the following procedures:

**Boiled bracts and hemp extracts**—Hemp dust or cotton bracts extracts were heated to 80°C in a boiling water bath for 30 minutes. The samples were centrifuged (3000 rpm) for 20 minutes and the supernatant adjusted to pH 7.4. These solutions were stored frozen at -20°C.

**Steam distillate of bracts extract**—A distillate of bracts extract was prepared as previously described.<sup>7</sup> Briefly, bracts extract was evaporated at 40°C under vacuum until about 80–90% of the original volume was collected as a distillate. Both the distillate and the residue were reconstituted to the original volume immediately before testing, with Tyrode's solution and deionised water, respectively.

**Charcoal treated boiled bracts extract**—Boiled cotton bracts extract (see above) was stirred with activated charcoal (Norit SG extra; 50 mg/ml) for two hours. The suspension was filtered and the pale straw coloured filtrate was stored frozen at -20°C. The charcoal residue was extracted with either methanol or boiling deionised water (60 min) to elute the adsorbed materials.

**Ether extract of boiled bracts extract**—A volume of boiled cotton bracts extract was saturated with sodium chloride and continuously extracted with ether in a light solvent extractor for 60 hours. The ether phase was separated and dried overnight with anhydrous magnesium sulphate. The dry ether phase was then evaporated at 50°C under nitrogen. The extracted materials were resuspended in Tyrode's solution in a volume equal to the original volume.

**Freeze dried boiled bracts extract**—Boiled bracts extract was freeze dried overnight (Lab ConCo, Freeze Dry 5). The residue was reconstituted in deionised water and Tyrode's solution to yield concentrations that were half, equal to, or double the original boiled bracts extract.

**Dialysed boiled bracts extract**—Double strength boiled bracts extract was subjected to dialysis. The extract was freeze dried overnight (Lab ConCo, at both ends. The sack was placed under running water overnight. The contents were removed from the sack and diluted before testing to a volume equivalent to single strength boiled bracts extract.

#### PREPARATION OF TISSUES

##### *Chopped lung tissue*

Female albino mice (DBA/2, Jackson Labs, Bar Harbor, Me, 10–20 g), rats (Sprague-Dawley, Charles River, Mass, 200–250 g), female guinea pigs (Hartley strain, Camm Research, Wayne NJ, 250–400 g) were killed by stunning and exsanguination. Monkeys (*Macacus rhesus*, Yale colony, 3 kg) were anaesthetised with pentobarbital sodium

(75 mg/kg, intraperitoneally) and exsanguinated via cardiac puncture. The lungs from the animals were rapidly removed and placed in cold Tyrode's solution. Samples of pig, cow, and horse lung were obtained at the time of slaughter from an abattoir. Human lung tissue was obtained at necropsy or from patients undergoing lung resection for carcinoma. Only lung tissue which appeared macroscopically normal was used.

Tissue samples were kept in chilled Tyrode's solution at all times, carefully cleaned of large bronchi and blood vessels, cut into thin slices (1 mm), and chopped using a McIlwain tissue chopper. Chopped particles were about 1 mm<sup>3</sup>. The chopped lung tissue was filtered on to a nylon mesh in a Buchner funnel and washed free of blood using at least one litre of Tyrode's solution. Excess fluid was then removed by suction. Weighed aliquots of lung tissue were used in the incubations (see below).

**Isolation of mast cells**—Mice and rats were lightly anaesthetised with ether and injected intraperitoneally with 5 ml and 20 ml of phosphate buffer respectively<sup>11</sup> to which human serum albumin (15% w/v) had been added (3 ml/l phosphate buffer). Mast cells were isolated according to the method described by Gillespie *et al.*<sup>12</sup>

#### HISTAMINE RELEASE AND ASSAY

Histamine release experiments were carried out using pooled, chopped, washed lung tissue or mast cell suspensions. Triplicate samples (0.1 g to 1 g of lung tissue depending on availability; 1 ml aliquots of mast cells about 10 ml) were incubated in Erlenmeyer flasks in a Dubnoff shaker at 37°C for 30 minutes in Tyrode's solution (5 ml final volume) under air. The tissues were incubated alone or in the presence of textile dust extracts (0.3 ml/ml, final flask concentration) or compound 48/80 (200 µg/ml, final flask concentration). In some experiments we incubated lung tissues with methyl piperonylate ( $5 \times 10^{-11}$  M to  $5 \times 10^{-5}$  M). Incubations were accompanied by reagent, vehicle, and extract controls in the assay procedure. A series of histamine concentrations (0.04, 0.08, 0.12, 0.16, and 0.20 µg/ml of incubate) were taken through the entire protocol in order to prepare a standard curve. Tissue and cell samples were also incubated with histamine (1 or 2 µg) to determine their metabolic activity. Incubations were terminated by filtration (lung tissues) or by centrifugation (mast cells) and an aliquot (2.5 ml) of the cell free supernatant was added to 30% w/v trichloroacetic acid (0.5 ml) to denature and precipitate protein. After centrifugation, the histamine content of an aliquot (2.5 ml) of the supernatant was determined by extraction and fluorometry.<sup>13</sup> Total histamine content of lung tissue was determined by

grinding 200 mg of chopped lung in 30% w/v trichloroacetic acid (0.5 ml). Total mast cell histamine was determined by adding 1 ml of suspension to 0.5 ml of 30% w/v trichloroacetic acid. After centrifugation, the histamine content of the supernatant was determined as described above. Histamine released into the medium was expressed as a percentage of the total histamine content.

#### HISTIDINE DECARBOXYLASE ACTIVITY

Albino mice (CFI special, Yale Colony, 10–15 g), in groups of three, were injected with aqueous extracts of textile dusts (retted and unretted flax and hemp, pericarps, bracts, and cotton). Four hours after injection (0.2 ml extract/mouse which was equivalent to 34 mg of textile dust), the mice were killed by decapitation and exsanguination. The histidine decarboxylase activity of the combined lungs from each group of three animals was determined by the method of Schayer *et al.*<sup>10</sup>

#### Results

There was no release of histamine into the medium after incubation of chopped lung tissues from mouse, rat, guinea pig, cow, horse, or monkey (fig 1) with any of the textile dust extracts (see methods). All of these tissues released considerable amounts of histamine when incubated with compound 48/80. Table 1 shows the metabolic activity of these tissues. Neither mouse mastocytoma cells nor rat peritoneal mast cells released histamine as a consequence of exposure to the extracts but did so when treated with compound 48/80 (data not shown). Only pig lung tissue released histamine when incubated with textile dusts (fig 2). Nevertheless, as with human tissues (see below), tissue samples from only half the animals studied (6/12) responded to the extracts. The remainder released histamine in the presence of compound 48/80 but not when incubated with textile dust extracts.

Human lung tissue, whether from surgical resection or from necropsy, showed the greatest release of histamine when exposed to textile dust extracts (fig 1). The mean total histamine content of the lung samples from either source was similar and thus a direct comparison of the data is valid. In general, the release of histamine was slightly greater from the necropsy lung tissues although the spontaneous release from these samples was also appreciably raised. Lung tissue from cadavers was unusable because of the large spontaneous release of histamine (data not shown) if more than 12 hours had elapsed since death. Compound 48/80 was the most potent releasing agent; the relative activities of the textile dust extracts were bracts > hemp > cotton

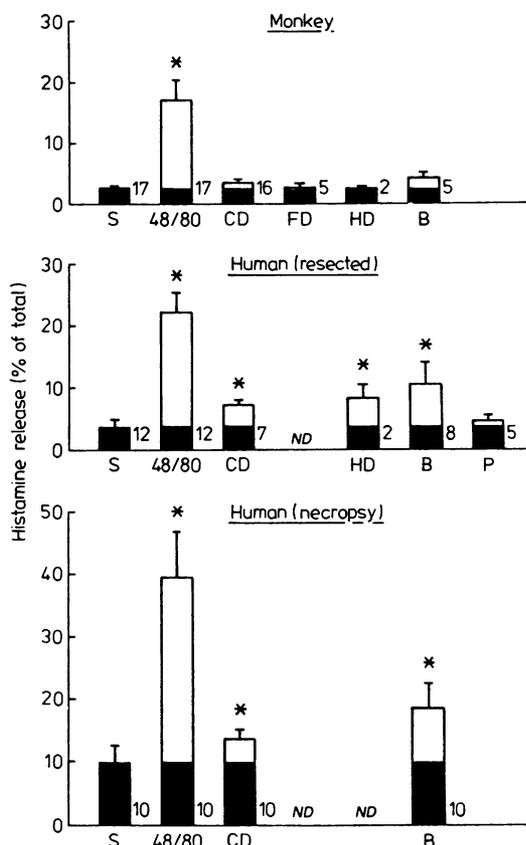


Fig 1 Histamine release from primate lung *in vitro*. Lung tissue was prepared as described in methods. Samples (500 mg or 1 g depending on availability) were incubated in Tyrode's solution (5 ml) at 37°C for 35 minutes alone (spontaneous release, S) or with compound 48/80 (final flask concentration 200 µg/ml) or textile dust extracts (1.5 ml equivalent to a final flask concentration of dust of 50 mg/ml). Textile dust extracts were cotton dust (CD), retted flax dust (FD), retted hemp dust (HD), cotton bracts (B), and pericarps (P). Histamine release is expressed as a percentage of total lung histamine content. Values are not corrected for metabolism of histamine that occurred during incubation nor for spontaneous release (shaded area). Numbers in bars are numbers of individual lungs examined. Values are mean ± SEM. \*Denotes release of histamine significantly greater than spontaneous release (Student's *t* test for paired variates,  $p < 0.05$ ). ND = Not determined.

dust > pericarp. While compound 48/80 always induced histamine release, the textile dust extracts were active in only 22 of the 40 lung specimens. Owing to the limited availability of human lung specimens our examination of modified textile dust extracts was carried out in pig lung tissue.

Table 1 Characteristics of lung tissue from various animals

Species (No)	Total histamine (ug/g)	Release with 48/80 (% of total)	Metabolism of histamine (% of 1 µg by 1 g)
Mouse (3)	1.6±0.1	ND	ND
Rat (22)	7.5±0.7	24.6±2.3	35.6±11.6*
Guinea pig (8)	32.6±2.1	3.2±0.4	15.3±3.5
Pig (6)	33.5±0.7	48.4±6.9	ND
Cow (3)	34.6±5.6	17.7±2.2	59.1±8.7
Horse (1)	31.3	6.6	ND
Monkey (17)	35.7±4.8	15.2±2.5	42.3±5.2
Human necropsy (10)	20.0±3.7	30.0±7.0	51.0±6.0
Human resected (12)	18.5±1.5	23.3±4.3	38.5±7.0

Values are mean ± SEM from (No) individual lung samples examined. Release data are not corrected for histamine metabolism but are corrected for spontaneous histamine release.

\*Metabolic activity was determined using 200 mg samples incubated for 30 minutes with 2 µg histamine.

ND = Not determined.

In these experiments samples of pig lung were incubated with several modified bracts extracts. Methyl piperonylate was inactive in pig lung tissue samples that released significant amounts of histamine when incubated with bracts extract (fig 2). Distillate of boiled bracts extract did not release histamine. Whereas the histamine releasing activity of

boiled bracts extract was removed when it was treated with activated charcoal, ether extraction did not remove the active constituent(s). Neither did freeze drying of boiled bracts extract diminish the histamine releasing capacity. The histamine releasing activity was not quantitatively related to the concentration of the extract used in the assay (fig 2). Table 2 shows histidine decarboxylase activity in mouse lung after treatment with the extracts. All the extracts produced appreciable increases in enzyme activity.

Table 2 Histidine decarboxylase activity of dust extracts

Extracts	HD Activity*
Saline	152
Bracts	697
Cotton dust	2120
Pericarp	489
Retted hemp	995
Unretted hemp	548
Retted flax	442
Unretted flax	835

Lungs of three identically treated mice were combined for each sample. All mice were killed four hours after an intraperitoneal injection of textile dust extract (0.2 ml). Dose was equivalent to 34 mg dust/mouse.

\*Histidine decarboxylase activity expressed as dpm C<sup>14</sup> histamine/100 mg lung tissue.

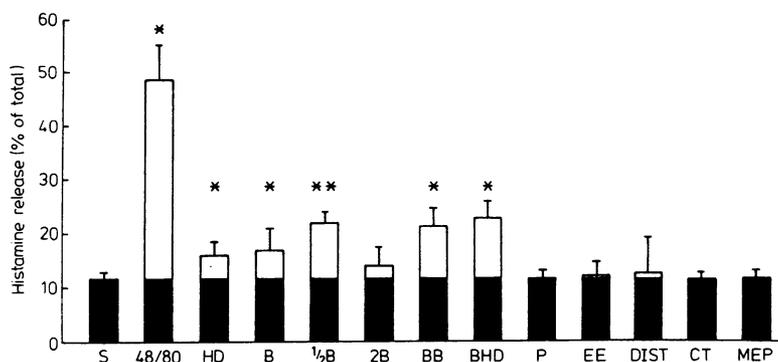


Fig 2 Histamine release from pig lung tissue *in vitro*. Chopped lung tissue samples (500 mg) were incubated in Tyrode's solution at 37°C for 35 minutes alone (spontaneous release, S) or with compound 48/80 or textile dust extracts.

Concentrations were as shown in fig 1. Textile dust extracts were retted hemp dust (HD), cotton bracts (B), boiled cotton bracts (BB), boiled retted hemp dust (BHD), and pericarps (P). 1/2B and 2B signify incubation with half strength and double strength boiled cotton bracts, respectively. In addition, histamine releasing abilities of fractions of boiled cotton bracts were examined (see methods)—namely, an ether extract (EE), a steam distillate (DIST), and a charcoal treated extract (CT). Methyl piperonylate (MEP) was also tested (see methods). Values are not corrected for metabolism of histamine that occurred during incubation nor for spontaneous release of histamine (shaded area). Histamine release was expressed as a percentage of total histamine content. Values are means ± SEM of six experiments. \*Denotes release of histamine significantly greater than spontaneous release (Student's *t* test for paired variates).

## Discussion

Our aim was to refine vegetable dust extracts in order to identify the active components and to find and use an assay procedure that was both quantitative and qualitative. Early experiments with chopped lung tissue and isolated mast cells from various animal sources showed that these dust extracts release histamine only from pig lung. The relative potencies of textile dust extracts in this tissue are similar to those in human lung tissue.<sup>3</sup> For these reasons and because of limited availability of human lung tissue, we chose pig lung as our assay tissue. Attempts to develop a quantitative assay based on the histamine releasing ability of cotton bracts extract were unsuccessful as shown by experiments with half, normal, and double strength extracts. The release of histamine was not concentration dependent (fig 2), possibly because of the presence of other substances in the extracts which augment or inhibit the release process, or both.<sup>5,6</sup> Until more highly refined extracts are prepared, therefore, the histamine release assay can be used only as a qualitative tool for the determination of active components of dust extracts.

A precise definition of byssinosis, which is caused by the inhalation of textile dusts, is complicated because some individuals show acute reactions to textile dusts and some develop chronic lung disease. It is not known whether all acute responders would eventually develop the chronic disease nor is it clear that all those with chronic disease showed acute response on first exposure. The mechanism(s) responsible for these functional lung changes are not known but three principle hypotheses have been proposed: (a) damage is induced by the inhalation of endotoxins present in vegetable fibres,<sup>9</sup> (b) antigenic sensitisation occurs as a consequence of exposure to components of the cotton plant,<sup>14</sup> and (c) histamine release is induced by components in textile dusts.<sup>15-17</sup>

Our data show that these dusts induce histidine decarboxylase but, unlike the data on histamine release described above, there is no correlation between their enzyme inducing activity and their bronchoconstrictor activity. For example, pericarps and unretted flax are two of the most potent enzyme inducers yet these agents are inert in the workplace or in laboratory tests in man. Similarly, antigenic sensitisation to components of these dusts does not occur over the short term in man as is shown by the fact that the severity of responses to repeated inhalation of the extracts remains constant.<sup>18</sup> Additionally, there is no change in the complement C3 titres in men chronically exposed to hemp dust.<sup>19</sup> In fact it would be difficult to reconcile these mechanisms

(antigenic sensitisation and enzyme induction) with reports that naive human subjects show acute responses to textile dust extracts and that this response can be prevented by pretreatment with an antihistamine.<sup>4</sup>

We have been able to confirm previous reports<sup>1</sup> that human lung tissue releases histamine when exposed to textile dust extracts. Our results show that the textile dust extracts active in man release histamine in vitro. We conclude, therefore, that methyl piperonylate cannot be the active component in cotton bracts extract. Additionally, this chemical does not cause bronchoconstriction in man.<sup>18</sup> It is noteworthy that not all lung tissues (from either man or pig) respond to these extracts. Whether there is a correlation between histamine release in vitro and the acute byssinotic response is uncertain.

We thank the staff of Bridgeport Hospital for their cooperation in obtaining specimens of human lung tissue.

Requests for reprints to: Dr James S Douglas, John B Pierce Foundation Laboratory, 290 Congress Avenue, New Haven, Connecticut 06519.

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- <sup>1</sup> International Steering Committee of Medical Editors. Uniform requirements for manuscripts submitted to biomedical journals. *Br Med J* 1979;1:532-5.
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