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Histamine release from platelets for assay of byssinogenic substances in cotton mill dust and related materials

S. K. AINSWORTH, R. E. NEUMAN, AND R. A. HARLEY

From the Department of Pathology, Medical University of South Carolina, Charleston, South Carolina, USA

ABSTRACT Previous reports suggest that byssinosis, an asthma-like condition among textile workers, may be mediated in part by histamine liberated following inhalation of dust. A simple, sensitive, and reliable procedure using pig platelets which contain the unusually high concentration of 0·8–1·6 μg histamine/10^9 cells has been devised for the assay of histamine-releasing factors in cotton mill dust and related materials, and has yielded results generally in accordance with earlier assays using chopped lung tissue. As little as 50–100 μg of total extractable substances from cotton mill dust can be measured. The activity of the extract is associated with the non-dialysable high molecular weight portion. However, conditions of acid hydrolysis do not destroy the activity. Extracts of leaves from different varieties of plant are highly potent, which suggests that the factors responsible for byssinosis are widely distributed plant components, present in textile fibre plants and converted to a respirable form by handling processes. Ellagic acid and sodium metasilicate release histamine from pig platelets, and represent new classes of compounds with possible roles in the aetiology of byssinosis.

Byssinosis affects as many as a quarter of the cotton workers in the United States, and is of increasing concern to the industry (Harris *et al.*, 1972; Bouhuys, 1974). The primary symptoms are respiratory difficulty, tightness in the chest, shortness of breath, cough, and decreased forced expiratory ventilation. The effects are most severe after absence from work (on Monday or after holidays) but gradually diminish as the work week progresses. The incidence and harshness of the attacks are related to the atmospheric concentration of respirable dust particles from the cotton materials. The disease eventually can develop into a chronic form with accompanying disablement (Harris *et al.*, 1972; Bouhuys, 1974).

Identification of the factor(s) in cotton mill dust which may be responsible for byssinosis could help to prevent or to alleviate this ailment. The nature of the symptoms and results of experimental studies suggest that induction of histamine release by cotton dust may be involved in the aetiology of byssinosis as observed in asthma (Bouhuys and Lindell, 1961; Bouhuys, 1974). Antihistamines are reported to allay development of the symptoms of byssinosis in experimental studies (Bouhuys, 1963; Valić and Žuškin, 1973). Increased quantities of metabolites of histamine are excreted following exposure to textile dusts (Bouhuys *et al.*, 1967; Edwards *et al.*, 1970). Agents in cotton mill dust and cotton bract extracts induced release of histamine from chopped lung tissue (Bouhuys and Lindell, 1961; Nicholls *et al.*, 1967; Evans and Nicholls, 1974b). On the basis of similar chemical properties these substances which release histamine apparently are identified as the bronchoconstricting agents of cotton dust *in vivo* (Douglas *et al.*, 1974).

A major problem in the study of the byssinogenic agents has been the question of suitable assay systems. These have ranged from inhalation by human volunteers (Hamilton *et al.*, 1973), leucocyte recruitment in the lungs of animals *in vivo* (Rylander and Nordstrand, 1974; Walker *et al.*, 1975), and chemotaxis in Boyden chambers (Kilburn *et al.*, 1977), to histamine release by chopped lung tissue (Bouhuys and Lindell, 1961; Evans and Nicholls,

Requests for reprints to S. K. Ainsworth, PhD, Department of Pathology, Medical University of South Carolina, Charleston, South Carolina 29403, USA.

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1974b). The latter method has been used in a number of productive studies. The rationale for the use of histamine release *in vitro* as an assay of byssinogenic agents can be based first, upon the evidence for the involvement of histamine in producing symptoms of byssinosis and second, on the repeated observation that extracts of cotton dust will induce histamine release in chopped lung tissue, as well as in the whole animal.

Unfortunately, in our laboratories and in others, the use of chopped lung tissues has appeared excessively laborious and not always reliable or sufficiently sensitive (Greenblatt, 1977). It occurred to us that leucocytes or, more precisely, basophils, might be employed satisfactorily in the detection of those substances in cotton dust that induce the liberation of histamine. While the mast cell is the localised source of histamine in the lung and other tissues, the blood-borne basophil in most species is the principal carrier of histamine in the circulation. Both mast cells and basophils contain histamine granules and respond to a number of the same agents that cause release of histamine (Cline, 1975). The specific release of histamine from sensitised basophils and tissue mast cells occurs in the presence of antigens (Mota, 1963; Lichtenstein and Osler, 1964).

In preliminary studies, pig leucocytes apparently did release histamine when treated with cotton mill dust extracts (Ainsworth and Neuman, 1977a). However, platelets which frequently persisted in leucocyte preparations were found to contribute significantly to histamine release, and to be superior as test cells. Although pig blood has a high histamine level (Lorentz *et al.*, 1971), the exceptional histamine content of platelets (0·8–1·6 $\mu g/10^9$ platelets or 80–90% of the blood histamine) has not been reported previously. The present report is concerned with the use of pig platelets to assay those agents in cotton mill dust or related material that may induce histamine release.

The use of histamine release from platelets for the detection and assay of possible byssinogenic agents, as in the chopped-lung assays, is based on the involvement of histamine as an effector mechanism in byssinosis, and on the fact that cotton mill dust extracts do release histamine from the platelets. Furthermore, results of the platelet assay method agree closely with published findings of chopped-lung assay techniques.

Material and methods

Samples of cotton mill dust (electrostatically precipitated using a lint prefilter) were kindly supplied by the USDA Cotton Quality Research Station Model Cardroom, Clemson University, Clemson, South Carolina. Gin trash was obtained from gins in Berkeley County, South Carolina, and cotton bracts and leaves were collected from cotton plants in the same area.

Extracts of cotton mill dust (CMDE), gin trash (GTE), cotton bracts and leaves were prepared by macerating in a mortar or shredding in a Waring Blender 5–15 g of material in 100 ml of water. After the suspensions had stood for 12–16 h at 4 °C, with occasional agitation, they were centrifuged at 2000 *g* for 30 min at 4 °C. The supernates were collected and stored at −10 °C. Before use, the extracts were thawed and recentrifuged for 10 min to remove a brown precipitate that had formed. Dialysates of extracts were prepared by immersing extracts in cellulose dialysis tubing for 24 h in 2 volumes of water at 4 °C. Dialysed residues were prepared by dialysing (dialyser tubing from Fisher Scientific Co, Pittsburg, Pa) the remaining contents of the tube against five changes of ten volumes of water at 4 °C over 72 h. Dry weights of extracts were determined by freeze-drying aliquots of the supernate and weighing the product. For hydrolysis, samples were heated in 6 M HCl in sealed tubes at 105 °C for 1–4 h. HCl was removed by drying the solution at 100 °C with a stream of air.

Pig blood was collected by needle puncture of the vena cava of an animal under restraint or, more usually, from the vena cava of pigs tranquillised by injection into the shoulder muscles of 10 mg ketamine HCl/kg body weight (Parke, Davis & Co, Detroit, Michigan). Yorkshire pigs aged 18 wk and weighing 50–60 kg were used. Platelets from blood obtained by either procedure showed the same histamine content and histamine-releasing activity.

Tris-buffered saline (TA) contained: tris (THAM; tris(hydroxymethyl)aminomethane: Fisher Scientific Co, Pittsburgh, Pa), 25 mmol/l; NaCl, 120 mmol; KCl, 5 mmol/l; and human serum albumin, (normal serum albumin (human) USP, 25% solution: Parke, Davis & Co), 1 mg/ml. TA with the further inclusion of 600 μmol Ca^{++}/l and 1 mmol Mg^{++}/l was designated TAC. The pH was adjusted with HCl to pH 7·65 at 25 °C (7·35 at 37 °C). Aqueous solutions of test samples were brought to the same basal composition of TAC by addition of concentrated stock solutions, and the pH was adjusted when necessary.

The procedure for determination of induced histamine release from platelets followed closely the method of Lichtenstein and Osler (1964) for determination of histamine release by sensitised human leucocytes in the presence of antigens. A major difference, of course, was the preparation of

platelets rather than of leucocytes. One hundred ml of pig blood was collected in a plastic vessel containing 11·0 ml of 0·1 M EDTA (ethylenediamine tetra-acetic acid, disodium salt) pH 7·7, as an anticoagulant and preservative of the platelets. After sedimentation at 4 °C for 1–2 h, the platelet-rich plasma was drawn off and centrifuged at 100 *g* for 10 min to remove suspended red blood cells and leucocytes. The supernate was then centrifuged at 1200 *g* for 15 min. The pellet of platelets was resuspended in cold (4–10 °C) TA. The cells were centrifuged and washed again. Finally the pellet was resuspended in 50–75 ml TAC. This cell suspension usually contained 5–9 × 10^8 platelets/ml and 0·4–0·8 μg histamine/ml. Platelets were counted with the Coulter Counter (Model ZBI, Coulter Electronics, Inc, Hialeah, Florida). Test samples (in TAC) were distributed in 12 × 75 mm tubes. The volume in every tube was made up to 1·0 ml with TAC, and 0·5 ml platelet suspension was added. Cell blanks were prepared with tubes containing TAC or sample solutions. The total histamine available from cells (designated 'cell completes') was determined from tubes containing cells and TAC which were treated directly with tricholoroacetic acid as described below, without previous removal of cells.

After the tubes had been incubated at 37 °C for 30 min with occasional agitation, the reaction was stopped by placing the tubes in a water bath at 4 °C. The cell-free supernatant collected from centrifugation at 1200 *g* for 6 min was mixed with an equal volume of 10% trichloroacetic acid. After 30 min at room temperature the tubes were centrifuged at 1000 *g* for 10 min. The clear supernatant was removed and its histamine content was determined by the fluorometric procedure of Shore *et al.* (1959). This procedure had been shown by Lorentz *et al.* (1971) to measure histamine accurately in all pig tissues tested, although whole blood extracts required additional treatment with Dowex 50 to eliminate interference that increased histamine results by 20%. However, the washed platelets in the present work had been separated from whole blood, and histamine results were not altered by use of Dowex 50. The Hitachi Perkin Elmer Fluorescence Spectrophotometer No MPF-2A (Perkin Elmer Corp, Norwalk, Conn) was used to measure histamine concentration. Results were expressed in terms of relative fluorescent reading (RFR) or as per cent net histamine release.

All pipettes and containers used to handle and incubate cells were plastic or coated with Siliclad (Clay Adams, Parssippany, NJ) to minimise adhesion of the cells. Cells and all solutions used were maintained at 4 °C throughout the washing process and preparation of tubes for incubation.

Results

Extracts of cotton mill dust and related materials induced release of histamine from pig platelets prepared and incubated in the manner described. However, in order to ensure optimal conditions, these were varied individually and the results were observed. Gin trash extract, because of its high histamine-release activity and ready availability in unlimited quantities, was used in much of the laboratory work reported here. In all studies, extracts of cotton mill dust and gin trash, which might be considered to be its immediate precursor, gave the same results.

The effects of altering the pH of the incubation medium are shown in Table 1 and Figure 1. Small quantities of histamine escaped from the platelets over the entire pH range. A net histamine release was observed on exposure to extract with maximal response between pH 6·8 and 7·4 (7·7 at 25 °C). Substitution of phosphate buffer for TAC gave similar results. Each reported value is the mean of results from three incubation tubes, and the precision

Table 1 *Effect of pH on histamine release from pig platelets*

*pH of incubator medium**	*GT extract (ml)*	*RFR†*	*Net RFR‡*	*Net release of histamine§ (%)*
6·1	0·0	3 ± 0·0¶	—	—
	0·2	48 ± 1·7	41	57
6·6	0·0	8 ± 0·5	—	—
	0·2	54 ± 0·7	42	58
6·8	0·0	7 ± 1·5	—	—
	0·2	57 ± 0·5	46	64
7·1	0·0	7 ± 0·0	—	—
	0·2	62 ± 2·0	51	71
7·4	0·0	7 ± 1·0	—	—
	0·2	58 ± 1·3	47	65
7·7	0·0	8 ± 0·5	—	—
	0·2	54 ± 1·0	42	58
8·0	0·0	8 ± 1·0	—	—
	0·2	48 ± 2·3	36	50
8·3	0·0	13 ± 2·0	—	—
	0·2	41 ± 2·3	24	33
Cell completes		72 ± 3·0		

*30 minutes' incubation at 37°C; †RFR: relative fluorescent reading; ‡Net RFR: RFR − (RFR of 0 tube + RFR of 4·0 for 0·2 ml GT extract); §Net release of histamine = $\frac{\text{net RFR}}{\text{RFR of cell completes}} \times 100\%$

¶Values represent the mean of 3 tubes, and the average deviation of each tube from the mean.

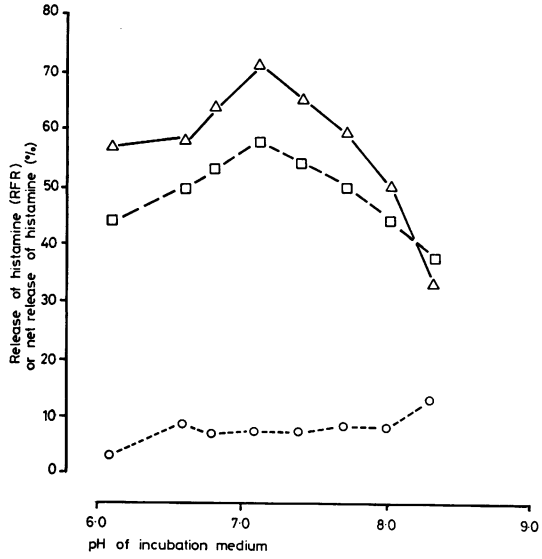

Fig. 1 *The effect of pH of incubation medium on release of histamine. Circles, 0 ml gin trash extract, RFR. Squares, 0·2 ml gin trash extract, RFR. Triangles, net histamine release, %. Incubation for 30 min at 37°C.*

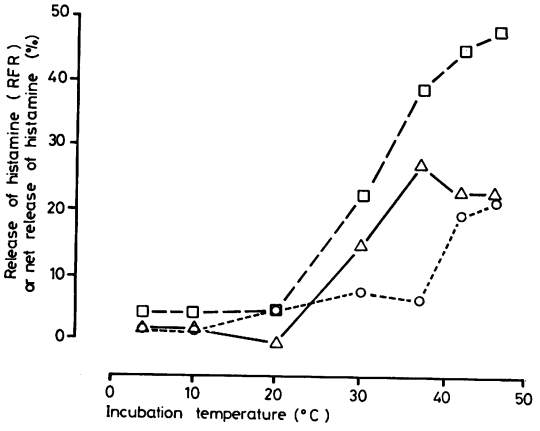

Fig. 2 *Release of histamine at different incubation temperatures. Symbols as in Fig. 1. Incubation for 30 minutes.*

of the experiment is expressed as the average deviation of the result from each tube, from the mean.

Figure 2 shows the effect of variation in temperature of incubation. The spontaneous release of histamine remained low at 4–37 °C, and increased thereafter. Below 20 °C the extract did not stimulate a significant release. With a further increase in temperature, the extract elicited histamine release with a maximal net effect at 37 °C. Higher histamine values were obtained at 42–46 °C, but this was attributable to greater losses from untreated cells, and did not, therefore, indicate a net increase in histamine release.

Figure 3 shows histamine release throughout the incubation period. Untreated pig platelets lost small amounts of histamine which gradually increased over a period of 60 min. Histamine values from test platelets, after an initial lag, rose after 20–30 min to a maximum which remained fairly constant. Net histamine release was maximal at 30 min.

The results obtained with different concentrations of platelets in the histamine release assay are shown in Figure 4. Histamine found in the medium of untreated as well as treated cells, increased in proportion to the concentration of platelets. However, at each platelet concentration the net percentage histamine release, calculated from the ratio of histamine in the medium to the total available from the platelets (cell completes), was approximately the same at the different platelet concentrations.

Recovery of histamine added to the test systems is shown in Table 2. The low recovery of histamine from the TAC medium alone was consistent with 30% losses during extraction with organic solvents during the fluorimetric histamine determination. However, no further losses occurred upon incubation with extracts and platelets so that, essentially, complete recovery was achieved.

Table 3 compares the histamine released from platelets prepared from two different pigs in two different experiments. Graded quantities of identical samples of cotton mill dust and a dialysed freeze-dried gin trash extract were assayed. As little as 0·04 mg of the cotton mill dust extract and 0·09 mg of the freeze-dried extract per ml in terms of extracted material were detectable. Net histamine released up to values near 50% was proportional to the amount of test substances. Therefore, the amount of test materials required for 25% net histamine release, equivalent to approximately half the maximal release of histamine, was often used to compare the potency of samples. Duplicate assays of samples compared in this manner gave acceptably similar results.

Application of the pig platelet assay to a variety of samples gave the results shown in Table 4. The data in Tables 2 and 3 bear witness to the reliability of these values. The assays gave results which did not vary over more than a 10% range of the net histamine release. Extracts of cotton mill dust, gin trash, cotton bracts, and leaves all gave a maximal net histamine release of 31–74%. This effect was achieved by 0·7–3·3 mg of dry weight of extract/ml. Net release of 25% histamine required only 0·2–1·7 mg/ml. These quantities of extracts generally

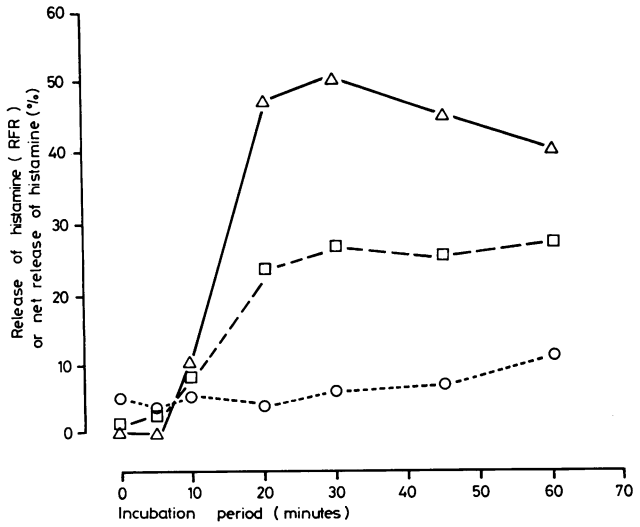

Fig. 3 *Release of histamine from platelets during the course of incubation. Symbols as in Fig. 1. Incubation at 37°C.*

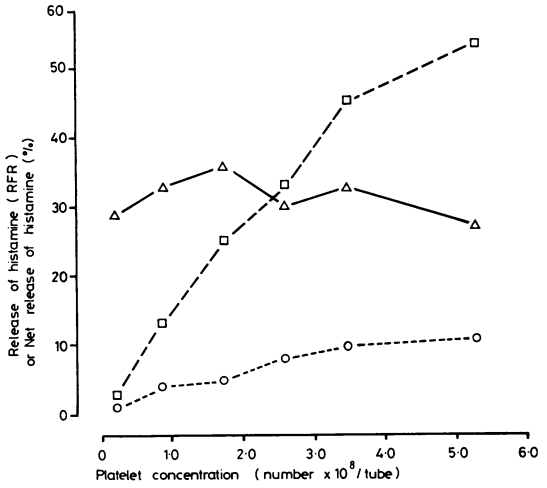

Fig. 4 *Relationship of concentration of platelets to release of histamine. Symbols as in Fig. 1. Incubation for 30 minutes at 37°C.*

represented 10–20% of the dry weight of the original samples.

The data in Table 4 show that extracts of leaves from other plant sources, the pecan and grape, are similar to the cotton extracts in their effects. Byssinosan prepared from gin trash following the procedure of Mohammed *et al.* (1971) and 'polyphenols' prepared from gin trash following the procedure of Taylor *et al.* (1971) were relatively inactive. Concentrations up to 10 mg/ml of human serum albumin, yeast RNA, adenosine triphosphate, lysozyme, dextran, methyl cellulose, glycine, alanine, histidine, arginine, glutamic acid and aspartic acid were inactive. Activities of dialysed residues of CMDE and GTE preparations were comparable to those of the original extracts, and the activity persisted after acid hydrolysis. Dialysates of CMDE and GTE were relatively inactive. Rutin and trimethylamine released histamine from pig platelets. Among tannin substances tested, quercetin and catechin (flavonoids of the condensed tannin group) and tannic acid (a hydrolysable gallotannin) showed low activity. However, ellagic acid, derived from a remaining group of hydrolysable tannins, the ellagitannins, did release histamine. Sodium metasilicate was found to have a similar high degree of effectiveness.

Discussion

The studies described here suggest that the conditions originally developed by Lichtenstein and Osler (1964) for the assay of histamine release with sensitised human leucocytes, also fall into the optimal range for the present assay of histamine release substances with pig platelets. Antweiler (1960) originally used animal blood to assess the histamine-releasing activity of cotton dust. Pig blood was used in the present studies because pig lung was known to be sensitive to cotton dust extracts and investigation showed that pig platelets had a

Table 2 *Recovery of histamine added to platelets and test media*

*Contents of incubation tube**	*Number of tubes*	*RFR*	*Histamine*		
			Found (μg/tube)	*Released*	*Recovered*
Without platelets					
Medium	2	0·0 ± 0·0†	0·000	—	—
Medium + 0·2 μg histamine	3	59·5 ± 0·5	0·141	—	0·141
Medium + 0·2 ml GTE	2	5·7 ± 0·4	0·015	—	—
Medium + 0·2 μg histamine + 0·2 ml GTE	3	67·7 ± 0·1	0·156	—	0·141
With platelets					
Medium	2	27·0 ± 1·0	0·064	—	—
Medium + 0·2 μg histamine	3	87·3 ± 4·7	0·207	—	0·143
Medium + 0·2 ml GTE	2	82·5 ± 2·5	0·195	0·116	—
Medium + 0·2 μg histamine + 0·2 ml GTE	3	144·0 ± 3·9	0·341	(0·116)	0·146

*30 minutes' incubation at 37°C; †Values represent the mean of 3 tubes, and the average deviation of each tube from the mean; 'Histamine found' represents value corrected for aliquots, but not for losses in extraction.

high histamine content and response.

This assay procedure provides a relatively homogeneous cell population that may be dispensed easily and reproducibly in replicate tubes by simple pipetting. The method is sensitive enough to enable 50% of the available histamine to be released by 3 mg of cotton mill dust, or by submilligram quantities of extract. In contrast, 100–200 mg of cotton mill dust has been necessary to release 5–15% of histamine from chopped pig lung. These differences are possibly attributable in part to mechanical barriers to diffusion in lung fragments. In both the platelet and the chopped lung assays, rutin and trimethylamine, found in cotton mill dust, and a high molecular weight fraction of cotton dust extracts (Evans and Nicholls, 1974a) induced histamine release. Previous studies (Ainsworth and Neuman, 1977a, b) showed that histamine-release activity was associated with high molecular weight fractions prepared from extracts by chromatography on Sephadex G-100, by ultrafiltration or by dialysis. However, despite acid lability of the factor determined by chopped lung assay, acid hydrolysates of the dialysed residues continued to cause histamine release from platelets.

Table 3 *Platelet histamine release from different pigs*

Equivalent of original sample (mg/ml)	*Soluble extract (mg/ml)*	*Net histamine release (%)*		*Requirement for 25% net release (mg/ml)*
		Pig no. 54	*Pig no. 55*	
Cotton mill dust extract no. 9B				
0	0	0	0	
0·07	0·022	6		
0·17	0·037	1 ± 0·02†	4 ± 0·6	
0·33	0·067	10 ± 0·05	9	
0·66	0·15	20	20 ± 1·4	
1·65	0·37	35 ± 0·4	46 ± 1·5	
3·3	0·72	60 ± 3·8	59 ± 0·9	(0·21 ± 0·01)
Dialysed freeze-dried gin trash extract				
0	0	0	0	
2	0·05		16 ± 0·9	
4	0·09	8 ± 0·5	26 ± 1·0	
8	0·18	21	33 ± 0	
20	0·45	37 ± 0	48	
40	0·9	47 ± 6·1	57 ± 1·1	(0·17 ± 0·08)

A platelet suspension was prepared from each pig. The same samples of CMD and GT extracts were assayed using different platelet suspensions, and a 30-minute incubation at 37°C.
†Values represent the mean of 3 tubes, and the average deviation of each tube from the mean.

Tannins are still under investigation in this laboratory as possible byssinogenic agents. Not only did several tannin compounds induce histamine release from chopped lung as well as from pig platelets, but they have chemical properties that may apply to the suspected histamine release factor(s) of cotton mill dust. Tannins might be expected to associate with other plant constituents, to form higher molecular weight polymers themselves, and to contain components that survive acid hydrolytic conditions (Haslam, 1966). Furthermore, as a prototype, a eucalyptus tannin fraction is extremely hypotensive in rats and apparently was the first tannin reported to have histamine-liberating activity (Read *et al.*, 1970). Moreover, ellagic acid was reported to release kinins and to lower blood pressure in the rat (Gautivik and Rugstad, 1967). The tannins are components of cotton mill dust and related materials, and are also widely distributed in the plant kingdom (Haslam, 1966; Taylor *et al.*, 1971). Byssinosis occurs in textile industries which use plant materials of diverse origin such as cotton, flax (Bouhuys *et al.*, 1961), hemp (Bouhuys *et al.*, 1967), and sisal (Nicholls *et al.*, 1973). These are subjected to the action of high-speed machinery, likely to produce particles of respirable dimensions which contain a common component of plants, such as tannins. Where wool or silk is the raw material, byssinosis is unknown. Presumably, wherever inhalation of suitably sized dust of plant materials

Table 4 *Histamine release induced by cotton mill dust extract and related materials in pig platelet assay*

Preparation or substance	*Maximum net histamine (%)*	*Quantity of test material* required for:*	
		Maximum net release (mg/ml)	*25% net release (mg/ml)*
Cotton mill dust extract (CMDE)	50	0·72	0·21
Gin trash extract (GTE)	74	3·3	0·30
Cotton bract extract	31	1·0	0·7
Cotton leaf extract	43	2·8	1·7
Pecan leaf extract	67	0·4	0·044
Grape leaf extract	58	1·5	0·11
Dialysed CMDE	49	1·2	0·20
Dialysed GTE	57	0·9	0·17
Byssinosan	6	3·0	—
Polyphenols	3	7·0	—
Acid hydrolysate of CMDE	62	1·7	0·54
Acid hydrolysate of GTE	41	0·9	0·31
Compound 48/80†	40	1·0	0·20
Rutin‡	46	1·0	0·18
Trimethylamine HCl‡	43	1·0	0·17
Quercetin‡	4	1·0	—
Catechin‡	6	0·6	—
Tannic acid§	17	1·0	—
Ellagic acid‡	43	0·6	0·16
Sodium metasilicate§ ($Na_2SiO_3 9H_2O$)	64	0·28	0·024

*Extracts in terms of freeze-dried weights; †Burroughs Wellcome Co; ‡Sigma Chemical Co, St. Louis, Mo; §Fisher Scientific Co.

containing histamine-releasing substances occurs, acute byssinotic symptoms are likely. However, physical irritation by particles and further biochemical effects could contribute to the production of symptoms, especially to the irreversible pathology of the chronic byssinotic syndrome.

Silicate is a second type of widely distributed substance that induces release of histamine from platelets. Silicon is a common constituent of plant tissues in the form of dissolved hydrous silica or silicic acids (Bailar *et al.*, 1973), which may contribute to the byssinotic syndrome. Silica has been reported to release histamine from lung *in vitro* (Religa and Malinski, 1970). Cotton bracts contain 0·4–0·8% silicon (Wakelyn *et al.*, 1977).

In the present study, the pig platelet assay of histamine-releasing substances is presented as a tool and not necessarily as a model system. It is not known at present whether platelets are directly involved in byssinosis. However, a reduction of platelets of cotton mill workers on the first day of the work week was reported by Bomski *et al.* (1971). An abundance of prostaglandins, serotonin and a quantity of histamine is present even in human platelets (Clausen and Srivastava, 1972; Seidel *et al.*, 1973). These compounds are bronchoconstricting substances, and prostaglandins and serotonin are taken up avidly by lung tissue (Adkinson, 1977). Furthermore, almost the entire platelet population of the blood traverses the lungs every few minutes. In certain asthma-like conditions (as in anaphylaxis of the guinea pig) bronchospasm and circulatory shock are mediated by histamine from platelets trapped in the lung (Aviado, 1976). Trauma in the dog leads to the accumulation of large numbers of platelets in the lungs, but not in other organs tested (Ljungqvist *et al.*, 1971). A special affinity of the lungs for platelets, rather than filter properties alone, was proposed as an explanation (Bergentz *et al.*, 1972). This hypothesis is not unreasonable despite the simpler interpretation that the histamine release from the platelets is an instance of the well-known release reaction of platelets in response to such substances as adenosine diphosphate, thrombin or collagen (Caens *et al.*, 1977).

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