

Analysis of environmental histamine from agricultural dust

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The agricultural worker can often be exposed to dust containing a complex mixture of organic materials. A variety of respiratory syndromes, including asthma, rhinitis, hypersensitivity pneumonitis, and reactive and chronic obstructive airway disease, has been associated with acute and/or chronic agricultural dust exposures. Dose-response relationships have not been established for any of the respiratory conditions and select dust etiologic agents. This lack of established association is probably due to the multiplicity of biologically active agents found in the agricultural environment. An analysis of substances or organisms that may modulate the response to the primary etiologic agent could possibly aid the understanding of the clinical sequelae that follow exposure to high levels of organic dusts.

Previous work in this laboratory has identified the presence of histamine in hay samples (1). Histamine is known as a potent vasodilator, bronchoconstrictor, and immunomodulator. The role of histamine in organic dust-induced disease is not known. It has the potential to cause, or contribute to, a primary response, as well as to modulate the response due to other agents such as endotoxin. A methodology to quantify histamine levels from environmental air and bulk samples has been developed in our laboratory. The assay is applicable to the monitoring of histamine exposure in the workplace.

Materials and methods

Sample collection and acid extraction. Representative samples of bulk materials were collected and placed in sterile plastic culture tubes. Air samples were collected on 37-mm, 0.45- μ m pore HA or DM filters (Millipore, Bedford, Massachusetts, United States). The filters were placed in sterile plastic tubes. Five milliliters of 0.1 M perchloric acid (HClO_4) per 250 mg of bulk or filter sample was added to each tube, vortexed, and then mixed on a rotator for 4 h. Supernatant fluids were recovered after centrifugation at $1000 \times g$ for 30 min.

Sample clean up. Two to 5 ml of the extracts were alkalized by the addition of 10 N sodium hydroxide (NaOH), 100 $\mu\text{l} \cdot \text{ml}^{-1}$, and immediately extracted into 0.75 ml of butanol. One-half a milliliter of the butanol was recovered after centrifugation and replaced by an equal volume of clean butanol, and the extraction was repeated. The total butanol extract recovered (1 ml) was added to 1.9 ml of heptane and back extracted into 0.2 ml of 0.12 N hydrochloric acid. A 0.19-ml aliquot of the hydrochloric acid was recovered and stored at -70°C until assayed. Histamine standards were run in parallel to the samples.

Radioimmunoassay. Aliquots of each sample and standard extract were neutralized by the addition of equal volumes of NaOH (~ 0.11 N) and diluted one-fifth to one-hundredth. The samples and standards were then acylated and assayed according to the manufacturer's instructions (AMAC, Westbrook, Maine, United States). Samples with histamine concentrations above the range of the standard curve were further diluted before or after acylation.

Culture of microorganisms. Cultures of bacteria and fungi were revived from lyophil preparations, and a fresh agar slant of each organism was used to prepare inoculum. Bacterial inoculum was prepared on tryptic soy agar (TSA, Difco, Detroit, Michigan, United States), and cultures were incubated for 24 h at 24°C in susceptibility test medium (STM). We prepared inoculum for fungi by growing the organisms on either Yeast-malt agar (yeasts) or malt extract agar (filamentous fungi) at 24°C for 5 d.

To test for histamine production, we incubated the organisms at 24°C in a shaker-incubator at 150 revolutions/min in 50 ml of medium in 125-ml Erlenmeyer flasks, 24 h in STM for the bacteria and 120 h in Czapek-dox broth for the fungi. After incubation, the contents of each flask were centrifuged in a 50-ml conical centrifuge tube at $1000 \times g$, the supernatant fluid was filtered through a 0.45- μ m cellulose acetate Millipore filter, and the filtrate was stored at -80°C until the analysis.

Results

Two extraction media, distilled water and 0.1 M HClO_4 , were evaluated for the extraction of his-

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mine from bulk and filter samples. Table 1 lists the percentage of recovery of ^{14}C -histamine added to 250-mg agricultural samples when extracted with distilled water or 0.1 M HClO_4 . The favorable partitioning effect of the acid extraction media improved the recovery of histamine from 13.6 to 45.8% over distilled water.

The crude aqueous acid extracts were cleaned up by extraction into butanol and then back into acid. The extraction efficiency of histamine into butanol and then back into the acid has been previously reported as 82% by analysis with high-performance liquid chromatography (2). Histamine extraction efficiency, as determined by monoclonal radioimmunoassay (RIA), was 84%. Standard curves obtained from extracted standards were comparable to that obtained with normal standards. This finding would suggest that any residual butanol in the buffered back-extract did not affect the RIA. Butanol-acid extraction also removes greater than 90% of acylhistamine, a possible interfering substance.

Air and bulk samples of hay, chicken coop bedding, rafter dust, wood mulch, silage, and cotton dust have been assayed, and all of them demonstrated measurable levels of histamine. Hay and samples from chicken coops have been extensively evaluated. The bulk hay samples ranged from 0.078 to 125.75 pmol histamine $\cdot \text{mg}^{-1}$ hay ($N=10$). The amount of histamine per weight of hay was greater in airborne dust than in bulk hay [116.74 (SD 346.1) pmol $\cdot \text{mg}^{-1}$ as compared with 11.45 (SD 31.06) pmol $\cdot \text{mg}^{-1}$]. The chicken coop samples displayed similar concentrations of histamine.

Bacteria and molds isolated from environmental and clinical sources or obtained from culture collections were cultured in defined media. These media were assayed for the presence of histamine. Table 2 presents a list of microorganisms shown by RIA to produce histamine under the culture conditions used. Histamine-producing organisms included both gram-negative and gram-positive bacteria, as well as fungi, including *Cladosporium cladosporioides*, *Penicillium brevicompactum*, *Acremonium species*, *Candida sake*, and *Cryptococcus laurentii*.

Discussion

Authentic histamine can be found in a variety of agricultural dusts. There are several sources that can contribute to this contamination. Samples from animal housing facilities, such as chicken coops, may contain histamine derived from animal excrement or insect infestation. These animal sources, however, cannot explain the presence of histamine in other agricultural dust that has no indication of animal contamination. On the other hand, levels of microbial contamination are usually high in these samples, and it is reasonable to expect that microorganisms could be major contributors. Of a total of 41 different isolates screened for histamine production, several bacteria,

Table 1. Extraction efficiency of bulk or filter samples of histamine.

Sample	Water	Extracted perchloric acid (%)
Cotton dust	47.7	68.3
Hay	66.2	79.8
Silage	19.7	65.5
Chicken coop bedding	29.3	55.1
Chicken coop rafter dust	..	48.3
HA filter	..	95.5

Table 2. Microorganisms that produce histamine.

Organism	Source
<i>Escherichia coli</i>	Clinical isolate, indoor air
<i>Cladosporium cladosporioides</i>	Rice dust
<i>Acremonium species</i>	Hay dust
<i>Candida sake</i>	Wood dust
<i>Cryptococcus laurentii</i>	Wood dust
<i>Penicillium brevicompactum</i>	NRRL 864
<i>Yersinia pseudotuberculosis</i>	Hay
<i>Clavibacter mighaganese</i>	Poultry farm dust
<i>Streptomyces species</i>	Oil

and fungi produced a substance recognized immunologically as histamine. The production of histamine may be dependent upon specific culture conditions, and, therefore, no claim is made that those organisms which were negative for histamine in our studies are incapable of producing histamine. For this reason, only cultures positive for histamine production were listed in table 2. For example, of the four different isolates of *Escherichia coli* tested, only two produced histamine.

We have evaluated several techniques for the extraction of histamine from environmental samples. HClO_4 extraction, followed by the butanol-acid clean-up technique, proved to be reliable for assaying agricultural or environmental bulk and air samples. This method cleans up the extract, removing possible interferences, and concentrates the samples. The extract is subsequently quantitated by RIA or chromatographic techniques. Either quantitative assay is applicable to the measurement of histamine from large batches of samples. The effect of exposure to agricultural dust histamine is unknown. Respirable concentrations of histamine resulting from the operation of chopping bedding have been measured and have been found to be as high as 10 nmol $\cdot \text{m}^{-3}$. Chicken catchers would probably be exposed to even higher levels since their work is a dustier operation. Histamine challenges that produce direct bronchial smooth-muscle contraction have been reported in the milligram per cubic meter range. This result would suggest that the environmental levels of histamine, by themselves, are not adequate to contract airway smooth muscle. It is not known if their effect would be additive or synergistic to other smooth-muscle agonists present in the dust. Histamine can also inhibit many cellular immune functions

through the H₂ receptor at concentrations many orders of magnitude less than that required for smooth-muscle contraction. The histamine exposure may possibly modify the inflammatory response of the lung to agricultural dust. The role of histamine in the acute pulmonary response to agricultural dust remains an enigma and may be important to the understanding of agricultural dust-induced disease.

References

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