Evidence for Histamine in the Urticating Hairs of *Hylesia* Moths*

Scott M. Dinehart, M.D., Joseph L. Jorizzo, M.D., Nicholas A. Soter, M.D., Nopadon Noppakun, M.D., William R. Voss, D.V.M.,† James A. Hokanson, Ph.D., and Edgar B. Smith, M.D.

Department of Dermatology (SMD, JLJ, NN, EBS), the Animal Care Center (WRV), and the Biostatistical Component, Cancer Center (JAH), The University of Texas Medical Branch, Galveston, Texas; and Department of Dermatology (NAS), New York University School of Medicine, New York, New York, U.S.A.

An urticarial dermatosis after contact with the urticating hairs of the adult female *Hylesia* moth may occur by several mechanisms including the intradermal injection of inflammatory mediators through the urticating hairs. Extracts were prepared from whole moths, urticating hairs, and other moth parts. Each of these extracts was subjected to a radioenzyme assay for histamine. Histamine was present in extracts made from whole moths and from urticating hairs. Extracts made from other moth parts contained no histamine. Cutaneous wheals occurred after intradermal injections of histamine and various concentrations of *Hylesia* extract (HE) into the backs of cynomolgus monkeys. This whealing response was suppressed by pretreatment of the animals with diphenhydramine hydrochloride, but not by pretreatment with indomethacin. Histologic examinations showed a perivascular lymphocytic infiltrate around dilated capillaries without evidence of mast cell degranulation in HE-injected sites but not in controls. These findings provide evidence that histamine may be the mediator responsible for the urticarial lesions seen after contact with *Hylesia* moths. *J Invest Dermatol 88:691–693, 1987*

Dermatitis occurring after contact with urticating hairs of the adult female *Hylesia* moth has been well documented [1–3]. The eruption is pruritic, occurs on exposed surfaces, and is commonly urticarial. The condition is generally unresponsive to various topical and systemic medications; however, spontaneous resolution occurs in about one week, provided contact with moths or moth parts is discontinued.

Several mechanisms for the production of the dermatitis have been proposed and include intracutaneous injection of toxic substance(s) through the hollow urticating hairs, direct irritant effect of the hairs, and hypersensitivity to insect antigen(s) [1,3]. More than one mechanism may be involved.

Dermatitis produced by *Hylesia* moths is frequent in Latin America but rare in the United States. We recently had the opportunity to examine crew members from an oil tanker that was infested by swarms of *Hylesia* moths at the port of Caripito, Venezuela [1]. The presence of a predominantly urticarial eruption in most of the crew suggested the possible role of histamine as a toxic substance contained in the urticating hairs.

Methods for identifying and characterizing mediators of cutaneous inflammation have been described [4–6]. The present study examines the actions of extracts of *Hylesia* moths in monkey skin over a wide dose range in the presence and absence of an H_1-receptor antagonist, diphenhydramine hydrochloride, and the cyclooxygenase inhibitor, indomethacin, to indirectly assess the presence of mediator activity. Using a radioenzyme assay, we examined extracts of whole moths, wings and legs, and urticating hairs for the presence of histamine.

MATERIALS AND METHODS

Subjects Indirect mediator studies employed healthy adult cynomolgus monkeys (*Macaca fascicularis*).

Intracutaneous Injections After sedation with i.m. ketamine hydrochloride (Ketalar®) at a dose of 15 mg/kg, the backs of 4 monkeys were shaved, washed with isopropyl alcohol, and then injected intradermally using a 30-gauge needle with 50 μl of dilutions of *Hylesia* extract (HE), phosphate-buffered saline, pH 7.6 (PBS), histamine, and buffer consisting of 1.0 M sodium chloride and 25% ethylene glycol (EG-NaCl). Injections were carried out at 2.5-min intervals in a double-blind random fashion according to our published method [5,6]. Two perpendicular diameters of the wheal, including the longest axis and ignoring small pseudopodia, were measured between 5 and 7 min, when the wheal was fully developed. The size of the wheal was given as the mean of the maximal diameters. Wheals measuring less than 7.5 mm median diameter were not considered a response, as this was the

Manuscript received August 19, 1986; accepted for publication November 25, 1986.

*Read before the Southern Region of The Society for Investigative Dermatology, Inc., New Orleans, Louisiana, February 7, 1986 and at the Annual Meeting of The Society for Investigative Dermatology, Inc., Washington, D.C., May 3, 1986.

†Present address: University of Texas System Cancer Center, Science Park, Veterinary Resources Division, Route 2, Box 151-B1, Bastrop, Texas 78602.

Reprint requests to: Scott Dinehart, M.D., Department of Dermatology, The University of Texas Medical Branch, Galveston, Texas 77550.

Abbreviations:

ANOVA: analysis of variance
EG-NaCl: buffer consisting of 1.0 M sodium chloride and 25% ethylene glycol
HE: *Hylesia* extract
K-W: Kruskal-Wallis
PBS: phosphate-buffered saline, pH = 7.6

0022-202X/87/$03.50

mean diameter of the injection bleb. The ambient temperature was 22–24°C. Evidence for delayed hypersensitivity at the sites of cutaneous injection was assessed at 48 and 96 h.

Pharmacologic Treatment Baseline dose response curves of wheal response were obtained after injections as described above on day 1. All 4 monkeys received 3.0 mg/kg of the H_1-receptor blocker diphenhydramine hydrochloride i.m. the morning of the experiment on day 3. Four hours later, another 1.5 mg/kg i.m. dose was given. The experiment began 2 h after the second dose and lasted 45 min to 1 h. All 4 monkeys received 4.0 mg/kg of indomethacin orally the morning of the experiment on day 5. Four hours later, another 4.0 mg/kg oral dose was given. The experiment began 2 h after the second dose and lasted 45 min to 1 h. Dosages of diphenhydramine hydrochloride and indomethacin were given in accordance with guidelines for nonhuman primates.

Solutions Appropriate dilutions of injected substances were made up freshly and sterilized by an acrylic copolymer membrane filter 0.20 μm (Gelman Acrodisc® no. 4192, Gelman Scientific, Ann Arbor, Michigan). In each experiment, response to HE diluted 1:1, 1:2, 1:4, and 1:16 with PBS was assessed. The response to 0.05 mg histamine/50 μl was a positive control. The responses to 50 μl of PBS and EG-NaCl were negative controls. All solutions were measured at pH 7.5–7.6.

The HE had been prepared as follows: the bodies of each of 10 adult moths, the separated hairs, and the wings of 1 moth were weighed and minced in plastic tubes containing 1.0 ml of EG-NaCl [7]. After boiling for 10 min, the tubes were sedimented at 400 *g* for 10 min, and the supernatant fractions were frozen at −20°C.

Assessment of Histamine Duplicate aliquots were assessed for their histamine content with a radioenzyme assay [8,9]. One aliquot was incubated with an excess amount of diamine oxidase (Sigma Chemical Co., St. Louis, Missouri) for 30 min at 37°C after which its histamine content was determined.

Histologic Examinations After sedation with i.m. ketamine hydrochloride, skin biopsy specimens (3-mm diameter) were taken without additional local anesthesia from the center of induced wheals between 10 and 20 min after injection of HE. Control biopsy specimens were taken from the sites of injection of PBS, EG-NaCl, and from normal, uninjected skin from the monkey's back. Each specimen was examined for mast cells and mast cell degranulation using light and electron microscopy by published methods [5,6].

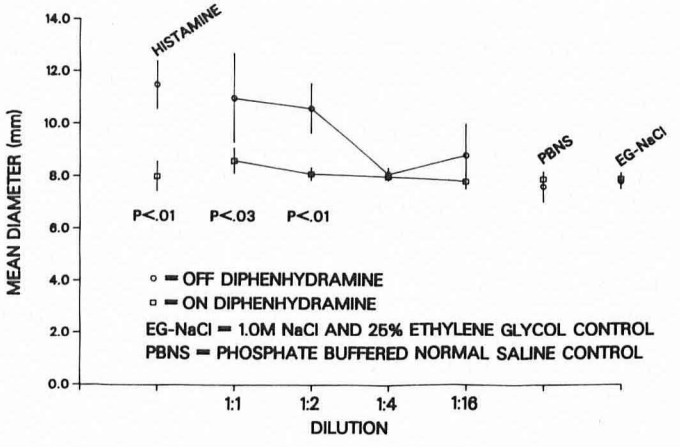

Figure 1. Dose response curves for the maximum diameter of wheals produced on the backs of cynomolgus monkeys by intradermal injection of various concentrations of *Hylesia* extract are shown without pharmacologic pretreatment (*circles*) and after pretreatment with diphenhydramine hydrochloride (*squares*). Histamine is a positive control. Phosphate-buffered saline and *EG-NaCl* are negative controls.

Statistical Analysis Analysis of variance (ANOVA) followed by contrast analysis were the primary statistical methods employed. The Statistical Package for the Social Sciences was used for all computations [10].

RESULTS

Local Effects in Monkey Skin Results of HE monkey injection experiments plotted on log 2 paper are summarized in Fig 1. Wheals produced by vehicle control injections approximated 8 mm, which was about the size of an injection bleb. Histamine injection wheals approached 12 mm. As the concentration of HE was increased, wheal size generally increased. After pretreatment with diphenhydramine hydrochloride, wheal formation was generally suppressed to the levels of control injections. For day 1 (no pharmacologic pretreatment) a one-way ANOVA was performed to compare PBS and EG-NaCl to the 4 dilutions of HE. Because these results were statistically significant ($F = 8.13$, $p < 0.01$), PBS and EG-NaCl were then compared with all the dilutions grouped together. As both the Bartlett-Box F and Cochran's C were not significant, the pooled variance estimate was used. The contrast analysis was statistically significant ($t = 3.37$, $p < 0.01$). These two analyses suggest that PBS and EG-NaCl produce values different than vehicle containing any amount of HE. Phosphate-buffered saline and EG-NaCl were compared with histamine of day 1. These results were statistically significant ($t = 48.8$, $p < 0.01$). These results were also consistent with the more conservative Kruskal-Wallis (K-W) procedure. For PBS and EG-NaCl vs dilutions of HE, the K-W chi-square was 13.0 ($p < 0.01$) and for comparison of PBS with histamine the K-W chi-square was 5.4 ($p < 0.02$).

The initial two-way ANOVA across days and dilutions indicated both significant main ($F = 17.5$, $p < 0.01$) and interraction ($F = 8.4$, $p < 0.01$) effects (Fig 1). Comparison of day 1 (no pharmacologic pretreatment) and day 3 (pretreatment with diphenhydramine) measurements indicated significant differences between no pharmacologic pretreatment and diphenhydramine pretreatment at the HE dilution of 1:2 ($t = 5.11$, $p < 0.016$) and for histamine ($t = 6.5$, $p < 0.01$) (Fig 1).

Pretreatment with indomethacin (day 5) did not have significant effect on wheal response to any dose of HE or to histamine. No evidence of delayed hypersensitivity at 48 and 96 h was seen at any injection site.

Histamine Content of Moths The histamine content (Table I) ranged from 7–38 ng per whole organism [mean 19 ± 6 (SD) and ± 1.9 (SEM)] and from 0.10–0.50 ng/mg wt [mean 0.24 ± 0.01 (SD) and ± 0.003 (SEM)]. Preincubation with diamine oxidase destroyed 100% of the histamine. Urticating hairs separately

Table I. Histamine Content in Extracts of *Hylesia* Moths

Moth Bodies	Weight (mg)	Histamine Content	
		ng/Whole Organism	ng/mg wt
1	78	29	0.37
2	78	23	0.29
3[a]	86	38	0.50
4	102	22	0.22
5	97	13	0.13
6	76	7	0.10
7	76	35	0.46
8	92	8	0.10
9	92	8	0.10
10	91	8	0.10
Mean		19	0.24
Standard deviation		6.0	0.01
Standard error of the mean		1.0	0.003
Hair	30	9	0.30
Wings	6	0	0

[a]After diamine oxidase: ng/whole organism = 0; ng/mg wt = 0.

analyzed contained 0.3 ng/mg wt. The wings did not contain histamine. Radioenzyme assessment for histamine was 70.6 ng/ml in the 1:1 HE.

Histologic Examinations

Light Microscopy: Control biopsy specimens from skin injected with PBS, EG-NaCl, and normal skin from the backs of the monkeys showed no abnormal histologic findings. Biopsy specimens taken from extract injection sites showed a perivascular lymphocytic infiltrate around slightly dilated capillaries in the upper papillary dermis. Endothelial cell swelling and mild perivascular edema were also present. No eosinophils were seen. Giemsa-stained sections did not show an increase number of mast cells or evidence of mast cell degranulation.

Electron Microscopy: Ultrastructural examination of sections from HE-injected sites showed a perivascular lymphocytic infiltrate around blood vessels in the papillary dermis. Degeneration of the endothelial cells of the capillaries was observed along with widening of gaps between endothelial cells. Some endothelial cells appeared to separate from the basement membrane. All mast cells examined appeared normal and were not degranulated.

DISCUSSION

Skin diseases resulting from contact with members of the order *Lepidoptera* (butterflies and moths) have been known since ancient times [11]. Many studies have been performed to isolate and characterize the agents responsible for lepidopteric dermatitis [7,12–15]. Some of these experiments have shown histamine to be present in the hairs or poison glands of certain species [7,12]. Ducombs et al suspected the presence of histamine in the hollow urticating hairs of the adult female *Hylesia* moth because of the appearance of wheals and a demonstration of increased vascular permeability after injection of an HE into the skin of guinea pigs [16].

Our studies confirm those of Ducombs et al that injection of extracts made from the urticating hairs of *Hylesia* moths causes cutaneous wheal formation. Additionally, we show that the size of this response is dose-dependent. The negative responses from extracting substances (PBS and EG-NaCl) eliminate the possibility that these solutions cause the response seen to HE.

The H_1-antihistamine, diphenhydramine hydrochloride, given in doses adequate to suppress histamine, significantly reduces wheal formation to HE at a concentration of 1:2. This finding supports our hypothesis that the whealing reactions to HE are at least in part mediated by histamine. The almost complete suppression of all cutaneous response to HE at higher concentrations by diphenhydramine suggests that histamine is the predominant mediator present in our extract. Although wheal response to 1:1 HE is large, we do not consider its suppression by diphenhydramine statistically significant, probably because of a large SD; the small number of subjects tested might have caused this high SD. The finding that indomethacin produces no significant effect on wheal formation suggests that products of arachidonic acid metabolism do not play a major role in the production of *Hylesia* dermatitis.

Radioenzyme assay has been shown to be a sensitive and specific method for the identification of histamine [8,9]. This assay showed histamine in extracts made from the urticating hairs of *Hylesia* moths (Table I). Histamine was found in much greater quantities from extracts made from the urticating hairs than extracts made from other moth parts, suggesting that the majority of the histamine found in *Hylesia* moths is present in the urticating hairs. The presence of histamine in the extract made from urticating hairs suggests that it may play an important role in the production of *Hylesia* dermatitis.

Light and electron microscopic studies of biopsy specimens did not show mast cell degranulation. The histologic findings of vasodilation, widening of endothelial gaps, and perivascular edema seen in specimens injected with HE are consistent with the intracutaneous effects of the injection of histamine into normal skin. One interesting finding is the presence of endothelial cell degeneration, which may represent a toxic effect of the extract. The discovery of endothelial cell degeneration was focal in nature and not associated with clinical purpura.

We have provided evidence that histamine participates in the production of *Hylesia* dermatitis. Other mediators or mechanisms are not excluded. Persistence of the eruption clinically, despite rigorous antihistamine therapy, suggests that other mechanisms of pathogenesis may be involved. It is known that the urticating hairs can penetrate and remain in the skin for long periods of time and possibly cause irritation by mechanical means [1]. Urticating hairs were filtered from our extracts prior to injection.

We thank Regina Scotto-Chinnici for expert technical assistance and Pat Moreno for typing the manuscript.

REFERENCES

1. Dinehart SM, Archer ME, Wolf JE, McGaveran MH, Reitz C, Smith EB: Caripito itch: dermatitis from contact with *Hylesia* moths. J Am Acad Dermatol 13:743–747, 1985
2. Hill WR, Rubenstein AD, Kovacs JR: Dermatitis resulting from contact with moths (Genus *Hylesia*). JAMA 138:737–740, 1948
3. Zaias N, Ioannides G, Taplin D: Dermatitis from contact with moths (Genus *Hylesia*). JAMA 207:525–527, 1969
4. Greaves MW, Black KA: Identification of mediators of inflammation, in Investigative Techniques in Dermatology. Edited by R Marks. St. Louis, Blackwell Scientific Publications, 1979, pp 94–103
5. Jorizzo JL, Coutts AA, Eady RAJ, Greaves MW: Vascular responses of human skin to injection of substance P and mechanism of action. Eur J Pharmacol 87:67–76, 1983
6. Coutts AA, Jorizzo JL, Eady RAJ, Greaves MW, Burnstock G: Adenosine triphosphate-evoked vascular changes in human skin: mechanism of action. Eur J Pharmacol 76:391–401, 1981
7. Shama SK, Etkind PH, Odell TM, Canada AT, Finn AM, Soter NA: Gypsy-moth-caterpillar dermatitis. N Engl J Med 306: 1300–1301, 1982
8. Beaven MA, Jacobsen S, Horakova Z: Modification of the enzymatic isotopic assay of histamine and its application to measurement of histamine in tissue, serum and urine. Clin Chim Acta 37:91–103, 1972
9. Shaff AB, Beaven MA: Increased sensitivity of the enzymatic isotopic assay of histamine: measurement of histamine in plasma and serum. Anal Biochem 94:425–430, 1979
10. Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH: SPSS: Statistical Package for the Social Sciences. New York, McGraw-Hill, 1977
11. Aber R, DeMelfi T, Gill T, Healey B, McCarthy MA, Oswell N, Ruhig W, Speziale H, Witte EJ: Rash illness associated with gypsy moth caterpillars—Pennsylvania. Morb Mortal Wkly Rep 31: 169–170, 1982
12. Valle JR, Picarelli ZP, Prado JL: Histamine content and pharmacological properties of crude extracts from setae of urticating caterpillars. Arch Int Pharmacol 98:324–327, 1954
13. Goldman L, Sawyer F, Levine A, Goldman J, Goldman S, Spinanger J: Investigative studies of skin irritations from caterpillars. J Invest Dermatol 34:67–79, 1960
14. Ziprokowski L, Rolant F: Study of the toxin from the poison hairs of *Thaumetopoea Wilkinsoni* caterpillars. J Invest Dermatol 46: 439–445, 1966
15. Ziprokowski L, Rolant F: Study of the toxin from poison hairs of *Thaumetopoea Wilkinsoni* caterpillars. J Invest Dermatol 58:274–277, 1972
16. Ducombs G, Lamy M, Michel M, Pradinaud R, Jamet P, Vincendeau P, Maleville J, Texier L: La papillonite de Guyane Francaise. Etude clinique et epidemiologique. Ann Dermatol Venereol 110:809–816, 1983