Evaluation of cutaneous responses and lung function from exposure to opiate compounds among ethical narcotics-manufacturing workers

Raymond E. Biagini, PhD, David M. Bernstein, MD, Stephen L. Klincewicz, DO,* Robert Mittman, MD,** I. L. Bernstein, MD,** and Gerry M. Henningsen, DVM, PhD** *Cincinnati, Ohio*

We recently demonstrated morphine-6-hemisuccinate-human serum albumin conjugate (M-6-HS-HSA)–specific IgG in serum from ethic narcotics-manufacturing workers. In this article, we present results of epicutaneous tests to opiate compounds and lung-function studies in these same workers. Thirty-nine workers, exposed to opiates, were evaluated for possible work-related changes in lung function and were administered a questionnaire concerning opiate exposure and health history in February 1988. In December 1988, 33 employees with occupational exposure to opiates, six other workers (New Jersey referent) employed at the same factory with minimal exposure to opiate compounds, and 17 nonexposed individuals from Cincinnati, Ohio, were subjected to epicutaneous threshold testing with a panel of six opiate compounds and nine common aeroallergens. In opiate-exposed workers, significantly lower epicutaneous threshold concentrations were detected (compared to New Jersey referent and Cincinnati control subjects) for dihydrocodeine ($p < 0.01$), hydrocodone ($p < 0.05$), codeine ($p < 0.01$), and morphine ($p < 0.05$). Significant associates existed among epicutaneous threshold concentrations between the agents tested; that is, individuals with a positive morphine skin test would generally have a positive codeine skin test, etc. Atopic status (positive cutaneous test results to two or more of nine common aeroallergens) was not significantly associated ($p > 0.05$) with positive opiate skin sensitivity. Although the mean cross-shift decrements in FEV_1 for all workers were nonsignificant, five opiate-exposed individuals demonstated cross-shift decrements in FEV_1 of >10%. Daily maximum-minus-minimum changes in workweek PEFR ($PEFR_{max\text{-}min}$) were significantly reduced for Monday through Thursday ($p < 0.05$) compared to $PEFR_{max\text{-}min}$ changes during a nonwork, nonexposure 3-day weekend. Ten exposed workers demonstrated daily $PEFR_{max\text{-}min}$ changes of >20%, suggesting acute airway obstruction. Increased cutaneous reactivity to opiate compounds among opiate-exposed workers may reflect development of pharmacologic hyperresponsiveness to opiate compounds. (J ALLERGY CLIN IMMUNOL 1992;89:108-18.)

Key words: *Hypersensitivity, asthma, morphine, codeine, dihydrocodeine, oxycodone, hydrocodone, antibodies, lung function*

From the Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Applied Biology Branch, Immunochemistry Research Section; *Division of Surveillance Hazards Evaluation and Field Studies, Hazards Evaluation and Technical Assistance Branch, Cincinnati, Ohio; and **The University of Cincinnati Medical Center, Department of Internal Medicine, Division of Immunology, Cincinnati, Ohio.

Presented in part at the Centers for Disease Control, 1989 Epidemic Intelligence Service Conference, Atlanta, Ga., April 1989, and at the Annual Meeting of the American Academy of Allergy and Clinical Immunology, Baltimore, Md, March 23-28, 1990.

Mention of a product or company name does not constitute endorsement by National Institute for Occupational Safety and Health.

Received for publication April 11, 1991.

Revised Aug. 6, 1991.

Accepted for publication July 26, 1991.

Reprint requests: Raymond E. Biagini, PhD, NIOSH, Applied Biology Branch, Immunochemistry Research Section, 4676 Columbia Parkway, Cincinnati, OH 45226.

1/1/33369

Pharmacologic health effects associated with the manufacture of opiate-containing drugs have been known for centuries, first reported by Ramazzini in 1715.[1] The mechanism of opiate-induced mast cell degranulation is controversial at the present time but is believed to be due to direct pharmacologic stimulation. There is experimental evidence that opiates induce granule depletion of human skin mast cells, and opiates (such as morphine and codeine) have been used in vivo as cutaneous mast cell-releasing agents.[2] However, the presence of mast cell opioid receptors, which could mediate these responses, have not been demonstrated conclusively.[2,3] Positive wheal-and-flare reactions to low concentrations of morphine are clearly antagonized by naloxone hydrochloride (a narcotic antagonist), whereas responses to higher morphine concentrations are not affected.[3] Antihistamines and antiallergic agents (astemizole or oxatomide) produce similar clinical inhibition of histamine-induced wheals, but there are differences in their effects on wheals elicited by morphine.[3] Histamine-induced wheal-and-flare responses are potentiated by met-enkephalin analogues β-endorphin and morphine, whereas vasoactive substances, such as substance P and vasoactive intestinal polypeptide, have no enhancing effect.[4]

There have been sporadic studies of asthmatic symptoms in opiate abusers,[5,6] and opiates have been demonstrated to have a variety of effects on the immune system.[7,8] Allergic contact dermatitis related to occupational codeine[9] and morphine[10] exposure has also been described; however, there has been no convincing evidence to support IgE-mediated allergic (immediate hypersensitivity) responses related to opiate exposure.

Animals that are immunized to morphine-protein conjugates readily produce IgG antibodies to morphine.[11-14] Antimorphine-specific IgE antibodies from parental morphine exposure have not been described in the animal literature.[14]

In February 1988, a NIOSH Health Hazards Evaluation was conducted on 39 opiate-exposed workers who were employed at a factory that extracts morphine and other related alkaloids from opium gum or related opium poppy *(Papaver sominiferans)* concentrates. During this initial survey, we performed daily and cross-shift lung-function measurements and administered a questionnaire to the workers. The questionnaire inquired about workers' general health status, symptoms, length of time working with opiates, whether they had ever been diagnosed as having asthma by a physician, and whether their symptoms were related to working with opiates. Based on general work practices and worker complaints from this initial survey, an improved RPP was advised for the workers, whereby half-face mask-cartridge respirators were substituted for less efficient particulate masks. In a follow-up survey done in December 1988, 33 workers (31 from the February 1988 survey) were evaluated for cutaneous sensitivity to opiates, and their sera were acquired for antibody analyses. Because of the 10-month time difference between Health Hazards Evaluation periods and the implementation of an improved RPP, associations between initial questionnaire results and PFTs and later skin tests and antibody levels could not be legitimately investigated. The results of specific opiate antibody analyses of sera from these workers have been published elsewhere[15] in which we demonstrated that exposed workers (before the implementation of the RPP) had significantly elevated levels of IgG antibodies to morphine. The goal of both these surveys was to establish the presence of immunologic or pharmacologic sensitivity to opiates in an attempt to define the pathogenesis of dermal reactions and airway obstruction from occupational exposure to opiate derivatives.

Abbreviations used

HSA:	Human serum albumin
CW_4:	Concentration yielding a 4 mm wheal diameter
NIOSH:	National Institute for Occupational Safety and Health
M-6-HS:	Morphine-6-hemisuccinate
M-6-HS-HSA:	Morphine-6-hemisuccinate-human serum albumin conjugate
PEFR:	Peak expiratory flow rate
$PEFR_{max-min}$:	Daily maximum-minus-minimum changes in PEFR
PBS:	Phosphate-buffered saline
LOD:	Limit of detection
PFT:	Pulmonary function test
RPP:	Respiratory protection program

MATERIAL AND METHODS

Process description and exposures

The manufacturing process at the plant included conversion of raw morphine base to United States Pharmacopeia powders, various morphine salts (hydrochlorides, phosphates, and tartrates), or derivatives of morphine, such as codeine and dihydrocodeine (didrate), oxycodone, hydrocodone, and similar compounds (Fig. 1, *structures*). The workers also had exposure to a variety of solvents, some of which possessed mild irritant properties (trade-secret con-

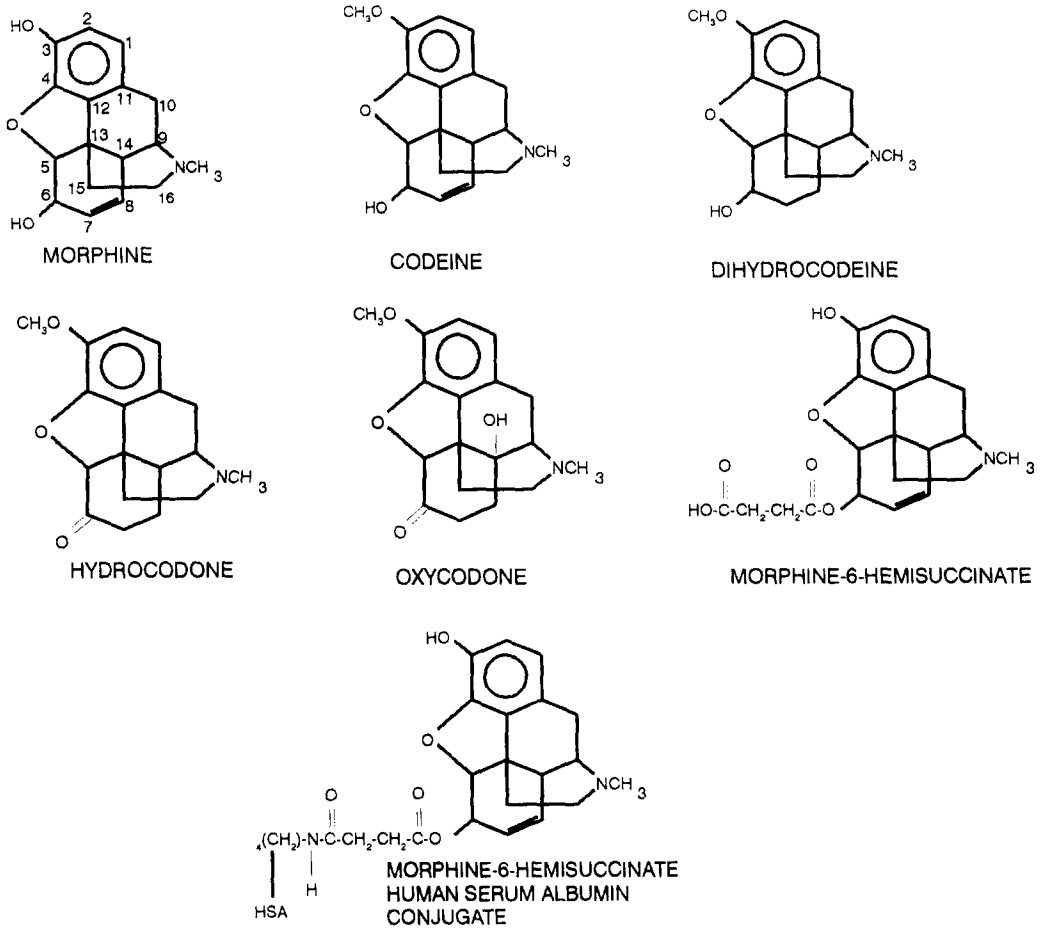

Fig. 1. Structure of opiate narcotics tested. Numbering system for morphine nucleus is illustrated on morphine alkaloid structure.

cerns prevent identification of specific solvents used); however, evaluation of industrial hygiene data and material safety data sheets failed to identify compounds, other than opiates, which could serve as an alternative explanation for respiratory complaints.

Environmental measurements

Twenty-seven short-term (7 to 54 minutes) and 12 full-shift personal breathing-zone samples for alkaloid dusts were collected by drawing air through 37 mm glass-fiber filters attached via tubing to a battery-powered pump at a flow rate of either 2.5 or 4.0 L/min (1 pm). Each sample was analyzed for codeine and morphine with high-pressure liquid chromatography.[27] Standards were first prepared by spiking known amounts of analytes onto glass-fiber filters. Samples and standards were then desorbed in 4 ml of mobile phase (0.01 mol/L of sodium pentane sulfonate in 22/78 acetonitrile/water) for 30 minutes with sonication. The resulting sample and standard solutions (150 μl in mobile phase) were injected into the high-pressure liquid chromatography system at a flow rate of 1.2 ml/min and analyzed at a wavelength of 254 nm. The LOD for codeine was 4.0 μg per sample, and the LOD for morphine was 5.0 μg per sample.

Study participants—General

The major complaints of the workers, which they believed were associated with occupational opiate exposure, included dyspnea, wheezing, headaches, malaise, tiredness, and skin reactions. Most evaluated employees were process workers; however, some employees worked in the laboratory and accountability positions that involved sporadic, lower, and less frequent opiate exposure. Process workers were exposed mainly during hand-scoop unloading of centrifuges, which sometimes proceeded without respiratory protection.

February 1988 participants

Thirty-nine current employees from the narcotics-production area participated in the study. No suitable comparison group was available because all individuals were potentially exposed to narcotic dusts. The exact number of employees in the building was considered a trade secret; therefore, the specific participation rate cannot be presented. The mean age of participants was 45 years (range, 23 to 63

years). Thirty-seven (95%) of the participants were male. The mean duration of employment in the building was 11.2 years (range, 0.3 to 36 years). Eleven (28%) employees were current smokers. Among current and former smokers, the average smoking history was 12.5 pack years (range, 0.1 to 78.8 pack years).

December 1988 participants

Thirty-one of the 39 previous participants agreed to participate in the December 1988 study. Of the eight individuals who did not participate, two current employees were ill, and one had difficulty scheduling a convenient time for the testing. Five of the original 39 participants, including one individual who indicated new-onset asthma, had terminated employement since the previous visit. Two additional current narcotic-production employees, including one other employee who indicated new-onset, physician-diagnosed asthma, were also enrolled in the study, even though they did not participate in the previous study. Thus, the December 1988 study participants included 33 current narcotic-production workers.

Eight managerial and secretarial employees with either low or no exposure to opiate substances served as the unexposed group (New Jersey referent). In addition, cutaneous tests performed in 17 volunteer control subjects from the Cincinnati, Ohio, area were included in the results of the present study. Although systemic opiate abuse was not specifically ascertained, none of the control subjects were suspected of its abuse.

Thirty-one of the 33 (94%) narcotic-production participants were male, as were seven of the eight (88%) referent workers. There was no statistically significant difference between the mean age of the narcotic-production workers and the reference-group participants (47 versus 46 years, respectively; mean ages of the Cincinnati cohort were not specifically ascertained but ranged from about 20 to 55 years). Informed consent was obtained from all participants in the present study, and the study protocol was approved by Human Subjects Review Board of NIOSH.

Antigen preparations

The workers were tested with a panel of nine common aeroallergens, including blue grass, elm, red oak, orchard grass, cat dander, *Alternaria*, *Cladosporium*, house dust, and short ragweed (Hollister-Stier Laboratories, West Haven, Conn.), in addition to five narcotic substances (codeine phosphate, morphine sulfate, oxycodone hydrochloride, hydrocodone hydrochloride, and dihydrocodeine bitartrate) and M-6-HS-HSA (Fig. 1, *structures*). All the narcotics were obtained from the manufacturing company and prepared as 10 mg/ml solutions in sterile PBS. M-6-HS was prepared by heating morphine (morphine alkaloid powder, United States Pharmacopeia) was succinic anhydride in pyridine as previously described.[10, 12] The pyridine was removed by reduced-pressure distillation, and the residue was washed with hot ethanol. The residue was then recrystallized twice from 60% ethanol (after refrigeration overnight at 4° C) to yield crystals that melted at 242° C to 243° C (uncorrected, with decomposition). Desorption probe-mass spectrometry identified the synthesized compound to be highly purified M-6-HS, since no extraneous mass ion peaks other than those caused by this compound were observed. The M-6-HS was then conjugated to HSA (albumin R, Armour Pharmaceutical, Kankakee, Ill.) by a mixed anhydride method.[11] After 4 hours, the solution was concentrated to 3 ml by use of a reduced-pressure dialysis apparatus (micro-ProDicon model 320, Bio-Molecular Dynamics, Beaverton, Ore.). The resultant concentrated M-6-HS-HSA conjugate was separated from any nonconjugated M-6-HS by gel filtration (Sephadex G-25M, Pharmacia, Uppsala, Sweden). Protein concentration was measured with a commercial Coomassie brilliant Blue assay kit (Bio-Rad Laboratories, Richmond, Calif.). The morphine content of similar M-6-HS-HSA conjugates was approximately 8 mol of morphine per mole of HSA.

Prick tests

For epicutaneous testing, 100 μl drops of test solutions were carefully placed on the skin and pricked with a 26-gauge hypodermic needle. After a period of 15 to 30 minutes, or when reactions were considered maximal, the areas were observed for obvious wheal-and-flare responses. Reactions were considered positive when there was a wheal with flare (the wheal being measured with a calibrated caliper and having a diameter not <4 mm at its widest point). bTesting was performed with five decremental serial tenfold dilutions. Skin test concentrations for all compounds ranged from 10^{-3} mg/ml to 10 mg/ml. If two dilutions produced identical 4 mm measurements, the result was recorded as positive at the higher dilution. Individuals who had a reaction <4 mm in diameter at 10 mg/ml were arbitrarily assigned positive scores at a dilution of 100 mg/ml. Histamine (0.1% histamine diphosphate, Eli Lilly and Co., Indianapolis, Ind.) was used as a positive control, and PBS (0.02 mol/L of phosphate buffer, pH 7.4, containing 0.9% NaCl) was used as a negative control.

PFTs

Preshift and postshift FEV_1 tests were conducted in a facility separate from the production area and were performed daily before and immediately after work. Two experienced examiners, certified by NIOSH in PFT procedures, conducted all examinations. Whenever it was possible, the same examiner performed all testing on an individual subject. Criteria for determination of adequate results are those described by the American Thoracic Society.[16] In addition to FEV_1 testing, each participant was provided with a mini-Wright spirometer peak flow meter for use during the entire week at work and at home. Each worker was intensely instructed in the use of the meter, and recorded the first reading on Monday morning before working in an area of potential exposure. These initial readings were performed in the presence of a physician member of the examining team. To monitor participants' self-recording of results, subjects met with the team each morning for each of the 5 days before work, submitted the sheet from the previous day for evaluation, and were issued new sheets. During the 3-day holiday weekend after the study (Febru-

ary), workers were provided with a 3-day supply of sheets. Each participant was instructed to complete three PEFR maneuvers every 3 hours while the participant was awake. The best result of the three efforts was recorded. Readings judged as obviously spurious by the physician interpreting the results were excluded from analysis.

Questionnaire

A questionnaire designed to ascertain information about health symptoms experienced by employees during the past 30 days was administered by a physician member of the examining team.

Statistical analyses

All statistical analyses were performed by tests that do not assume a normal distribution of experimental subjects, as we had no a priori knowledge of the underlying data distributions. Analyses were performed with a microcomputer and commercially available statistical analysis packages. Differences in CW_4 among groups (opiate-exposed, N.J. referent, and Cincinnati control subjects) were evaluated by Kruskal-Wallis's one-way analysis of variance, followed by Mann-Whitney U tests. Differences in prevalence data among the three groups (number of individuals with a positive skin test at any concentration) were investigated by contingency-table analysis (2 × 3 chi-square, followed by Fisher's exact tests). Individuals with positive opiate skin tests were stratified on the variables atopic status (defined as positive skin tests with any two of the nine common aeroallergens tested) and nonatopic status. Mann-Whitney U analyses were performed to compare these groups. Percent $PEFR_{max\text{-}min}$ results (calculated by evaluating the expression $[(PEFR_{max} - PEFR_{min})/PEF_{max} \times 100]$) were investigated with the analysis of variance techniques described above, with results of each day compared to the combined results from a nonwork, nonexposure 3-day weekend. Spearman's correlation (r_s) analysis was used to investigate the association between positive skin tests with the agents tested. A $\alpha = 0.05$ was considered statistically significant.

RESULTS

All participants demonstrated positive skin prick test responses to the histamine (positive control solution) and negative responses to PBS (negative control). Thirteen of 30 of the exposed workers had positive skin test responses to two or more of the common aeroallergen test substances and were thus diagnosed as atopic (data not presented). The frequency distributions for positive skin tests for M-6-HS-HSA, dihydrocodeine, oxycodone, hydrocodone, codeine, and morphine at five concentrations ranging from 10^{-3} to 10 mg/ml are presented for the three groups (opiate-exposed, N.J. referent, and Cincinnati control subjects) in Table I. These data clearly demonstrate the anticipated wide variability in cutaneous responses to opiates in the groups tested. The percentages of workers having positive cutaneous responses to the test substances are presented in Table II. Statistically significant differences ($p < 0.05$) in prevalence were observed for M-6-HS-HSA, dihydrocodeine, hydrocodone, and codeine; however, with inspection of Table II, it becomes obvious that most differences were between the N.J.-exposed and Cincinnati control subjects, with the N.J. referent group being indistinguishable from the N.J. exposed group for most of the substances tested. To further distinguish group differences based on sensitivity rather than raw prevalence, the average geometric mean CW_4 was calculated. These data are illustrated in Fig. 2. Statistically significant decreases in mean CW_4s were found for dihydrocodeine ($p < 0.05$), hydrocodone ($p < 0.05$), codeine phosphate ($p < 0.05$), and morphine sulfate ($p < 0.05$) for the opiate exposed group compared to the Cincinnati control subjects and N.J. referent groups simultaneously. No significant differences in CW_4 were observed for M-6-HS-HSA and oxycodone. In addition, no statistically significant differences ($p > 0.05$) in CW_4s could be discerned for the test substances when opiate-exposed workers were stratified on the basis of their atopic status (data not presented). There also were no significant differences in total serum IgE levels for the groups of workers (data not presented). Associations between positive tests with the different substances were investigated by correlating multiple positive tests when they existed in the same exposed individual. Statistically significant correlations were observed for positive skin tests to M-6-HS-HSA and oxycodone, dihydrocodeine and hydrocodone and codeine phosphate, and with morphine sulfate and codeine phosphate. These results are presented in Table III.

Ten exposed individuals (February) reported a previous diagnosis of asthma confirmed by their physician. One individual had childhood asthma that was no longer active. A second individual reported the onset of adult asthma that clearly began before working with opiates. Of the remaining eight workers, all reported asthmatic symptoms after beginning work with opiates. In addition, an in-depth review of all medical records indicated that two other individuals had suspected the onset of asthma subsequent to beginning work in the exposure area. Follow-up questioning of the individuals confirmed these findings as possible asthmatic cases. Thus, of the 39 individuals responding, 10 (26%) indicated a diagnosis on new-onset adult asthma since beginning work with opiates. Four of these workers reported the development of asthma within 1 year of beginning work.

TABLE I. Frequency distribution of opiate skin test responses

Opiate and sample population (No. of subjects tested*)	No. reaction†	Concentration (mg/ml)				
		10	1	0.1	0.01	0.001
M-6-HS-HSA						
N.J. exposed (33)‡	21	7	4	1	0	0
N.J. referent (8)§	7	0	1	0	0	0
Cincinnati group (17)‖	16	0	1	0	0	0
Dihydrocodeine						
N.J. exposed (33)	0	8	19	5	0	1
N.J. referent (8)	0	3	5	0	0	0
Cincinnati group (16)	3	9	4	0	0	0
Oxycodone						
N.J. exposed (33)	28	3	2	0	0	0
N.J. referent (8)	7	0	1	0	0	0
Cincinnati group (15)	14	1	0	0	0	0
Hydrocodone						
N.J. exposed (33)	0	11	18	4	0	0
N.J. referent (8)	2	3	3	0	0	0
Cincinnati group (16)	5	8	3	0	0	0
Codeine						
N.J. exposed (33)	1	4	18	10	0	0
N.J. referent (8)	0	2	4	2	0	0
Cincinnati group (13)	3	4	6	0	0	0
Morphine						
N.J. exposed (33)	4	6	12	11	0	0
N.J. referent (8)	1	2	4	1	0	0
Cincinnati group (12)	3	7	2	0	0	0

*Total number of skin tests does not necessarily equal the total number of workers who could be tested since some tests were not done in all individuals.
†Number of individuals not giving positive reactions at the highest concentration tested.
‡Process workers, opiate exposed.
§Management and secretarial employees with either nonexistent or minimal exposure to opiate substances.
‖Normal volunteer control subjects from the Cincinnati, Ohio, area.

TABLE II. Percent of total individuals tested demonstrating positive skin test reactions to opiate substances

Substance	Worker group						p
	N.J. exposed* (N = 33)		N.J. referent† (N = 8)		Control† (N = 12-17)		
	%	(No. tested)	%	(No. tested)	%	(No. tested)	
M-6-HS-HSA	36	(12)	13	(1)	6	(1)	<0.05
Dihydrocodeine	100	(33)	100	(8)	81	(13)	<0.05
Oxycodone	15	(5)	13	(1)	6	(1)	NS
Hydrocodone	100	(33)	75	(6)	69	(11)	<0.01
Codeine	97	(29)	100	(8)	77	(10)	<0.05
Morphine	88	(29)	88	(7)	75	(9)	NS

NS, Not statistically significant.
*Process workers, opiate exposed.
†Management and secretarial employees with either nonexistent or minimal exposure to opiate substances.
‡Normal volunteers control subjects from the Cincinnati, Ohio, area.

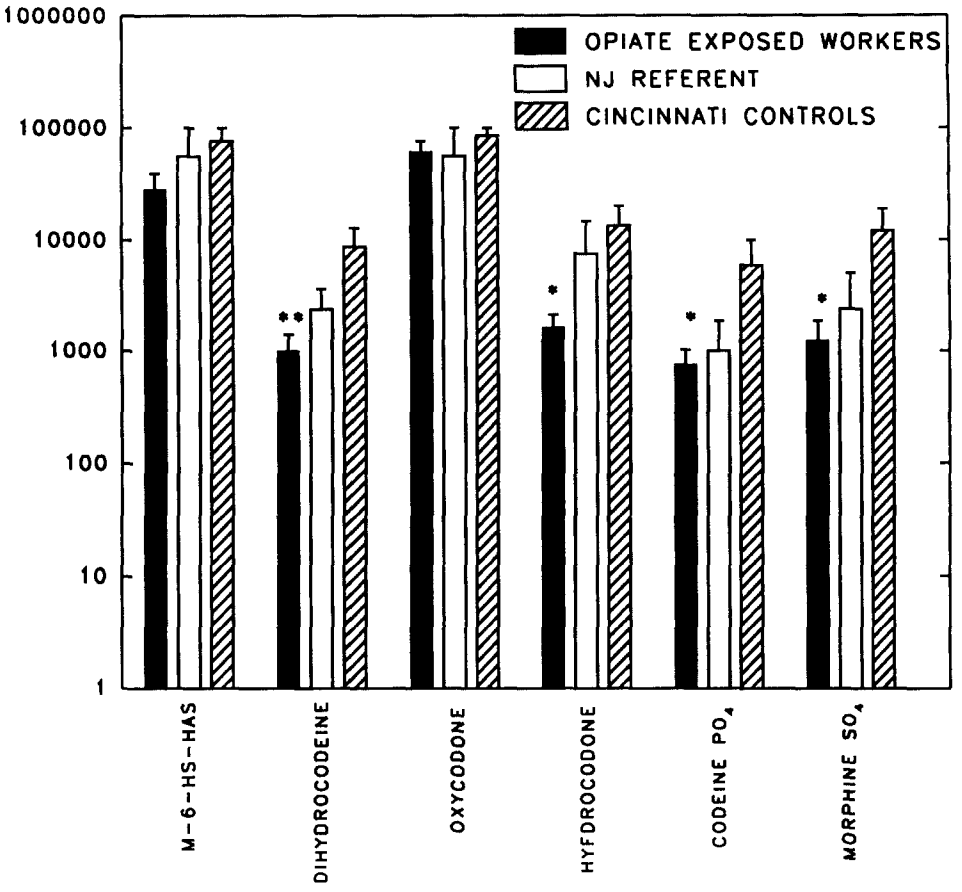

Fig. 2. Geometric mean concentrations of opiates producing 4 mm wheals in opiate-exposed, N.J. referent, and Cincinnati control individuals (**$p < 0.01$; *$p < 0.05$; see text for details).

TABLE III. Spearman's correlation coefficients for positive skin tests to a variety of opiate compounds in opiate-exposed workers*

Substance	M-6-HS-HSA	Dihydrocodeine	Oxycodone	Hydrocodone	Codeine	Morphine
M-6-HS-HSA	—	0.056	0.867†	−0.058	−0.078	−0.141
		(12)	(3)	(12)	(12)	(10)
Didrate	—	—	0.323	0.420†	0.333†	0.237
			(5)	(33)	(32)	(29)
Oxycodone	—	—	—	−0.166	0.166	0.166
				(5)	(5)	(5)
Hydrocodone	—	—	—	—	0.075	0.119
					(32)	(29)
Codeine	—	—	—	—	—	0.819†
						(29)

*The number of workers having skin test results at each matrix element are given parenthetically below their respective correlations.
†$p < 0.05$.

Twenty-four (62%) individuals reported a history of at least one episode of wheezing since employment at the factory. Of these 24 individuals, 21 (85%) reported episodes of work-related wheezing within the preceding month. Among the 21 individuals with wheezing during the preceding month, 17 (81%) reported that the wheezing occurred with shortness of breath, 14 (67%) with chest tightness, and 15 (71%)

with coughing. Six (29%) of the individuals reported that the episodes lasted less than 1 hour. Of the 21 individuals with wheezing during the past months, only one person indicated that episodes of wheezing occurred several hours after work exposure. When these individuals were asked how often wheezing followed certain exposures at work, responses were 10 (48%), most of the time; 7 (33%), sometimes; 2 (10%), rarely; 1 (5%), never; and 1 (5%), no response. Other reported symptoms at work included itchy, runny nose (49%), stuffy nose (57%), and itching eyes (56%). Sixteen individuals (41%) reported a work-related rash within the preceding 2 months. The distribution of the rash for those reporting it was hands (88%), face (56%), neck (62%), and forearms (75%).

Thirty individuals completed at least one set of preshift and postshift FEV_1 PFTs. Five individuals (two of whom had histories of adult-onset asthma) demonstrated cross-shift decrements in their FEV_1 of >10%. No significant changes ($p > 0.05$) in preshift versus postshift FEV_1 (either among days or between preshift and postshift measurements) were observed for these workers. Thirty-two of the 39 participants (February) completed all 8 days of $PEFR_{max-min}$ testing. Ten individuals demonstrated single-day decreases of >20% in $PEFR_{max-min}$, with three of these workers having histories of adult-onset asthma. When percentage changes in $PEFR_{max-min}$ were evaluated for all workers (February), statistically significant reductions were observed for Monday through Thursday compared to combined results for a 3-day nonwork, nonexposure weekend (Fig. 3).

Exhaustive correlation analyses of narcotic CW_4s with preshift versus postshift changes in FEV_1 or $PEFR_{max-min}$ in all workers or in workers dichotomized on having physician-diagnosed asthma since beginning work with narcotics were performed. The results of all these correlation analyses were uniformly nonsignificant ($p < 0.05$). It is believed that the limited number of workers meeting each criteria being correlated somewhat limited our power to detect statistically significant correlations.

Twenty-seven personal breathing zone short-term exposure samples for codeine and morphine were collected during seven different narcotic handling operations. Substantial airborne exposures were measured during operations that required the handling of dry alkaloid powder. Personal shortterm dust-sampling (range, 7 to 54 minutes) results ranged from below the LOD to 23.6 mg/m^3 for codeine, and from below the LOD to 10.5 mg/m^3 for morphine. The highest levels of both narcotics were observed during transfer of dry powders with hand-scooping techniques. Full-shift alkaloid concentrations were below the limit of

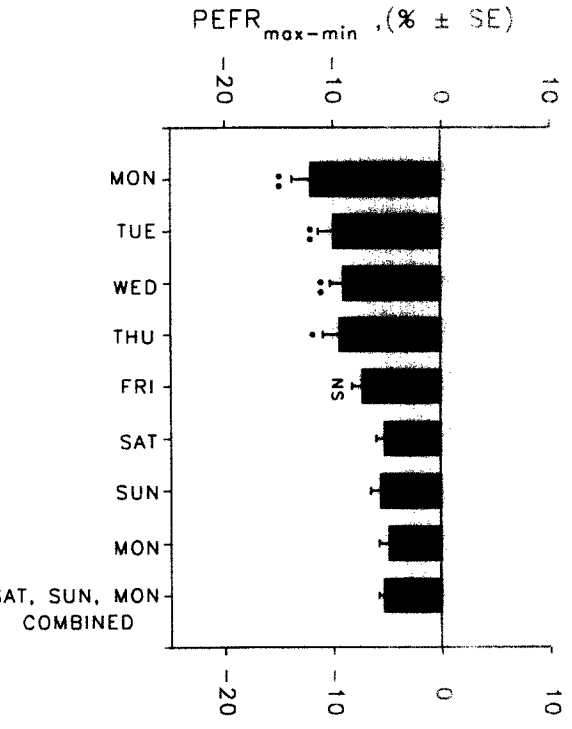

Fig. 3. Percent change in daily $PEFR_{max-min}$ in opiate-exposed workers compared to their combined $PEFR_{max-min}$ data for a nonwork, nonexposure 3-day weekend (N = 36; $**p < 0.01$; $*p < 0.05$; see text for details).

quantitation for morphine and ranged from below the limit of detection to 1.6 mg/^{3} for codeine.

DISCUSSION

In the present study, we have demonstrated significantly greater cutaneous sensitivity to a variety of opiate compounds in workers employed at an ethic narcotics-manufacturing facility, compared either to opiate-nonexposed factory control subjects or normal nonexposed volunteers. It is interesting to note that, when the data were analyzed as raw prevalence (proportion of workers with a positive test at any test concentration), statistically significant, but perplexing (i.e., most differences were between the N.J. exposed and Cincinnati control subjects, with the N.J. referent group being indistinguishable from the N.J. exposed group for most of the substances tested) results were obtained (Table II). When skin sensitivity was analyzed by a method that took into account the concentration of substance needed to yield a 4 mm reaction, in most cases the N.J.-exposed groups were significantly different from both the N.J. referent and Cincinnati control subjects. These findings reinforce the need to analyze skin test data by sensitivity rather than by raw prevalence in epidemiologic studies, especially for agents such as opiates that cause wheal and flare

in both control and exposed individuals. The findings of correlations between positive skin tests with the various agents (Table III) were not surprising, since the opiate compounds tested generally share a common morphine nucleus. Pulmonary function measurements suggested an association (albeit, nonsignificant) between workplace exposure to opiates and pulmonary function decrements (Fig. 3).

The ability of opiates to release histamine directly from mast cells has been well documented,[2] whereas mechanisms to explain regulation of mast cell responses to the opiates are poorly described. In a recent study of patients with systemic mastocytosis, case patients revealed no greater mean sensitivity to morphine than a group of control subjects.[3] Human mast cells have been demonstrated to vary widely in functional response to opiates.[3, 4] Such diversity in mast cell response has been putatively ascribed to the varying presence of specific opiate receptor sites on the cell membrane.[3, 4]

Frequently, idiosyncratic (including bronchospasm, hives, and flushing) reactions in patients receiving opiates have been termed "pseudoallergic reactions" because of their similarity to immunologically mediated reactions.[17] To date, no convincing human or animal data have revealed evidence of specific IgE or IgG4 (short-term sensitizing IgG) to codeine or morphine, although specific IgG to morphine has been demonstrated in the workers of the present study and in heroin drug abusers.[15]

Delayed-type hypersensitivity responses (contact dermatitis) have been reported among opiate-exposed workers in Spain.[9] Demonstration of IgG class antimorphine antibodies in animals[11-13] and humans[15] provide evidence that opiates are capable of producing specific immunologic changes. Finally, autologous and homologous anti-idiotypic anti-anti—β-endorphin IgG class immunoglobulins have been described[18] that compete with β-endorphin for opiate receptors, possibly regulating neuropeptide activity or pharmacologic activity of exogenous opiates. Binding of opioid neuropeptides by antimorphine antibodies or the production of antiantimorphine antibodies is also a consequence that could interfere with functions of endogenous neuropeptides. It remains speculative whether the release of histamine and other vasoactive substances from the mast cell might be altered by specific numbers or types of opiate receptors on mast cells and, furthermore, whether the presence or absence of such antibodies could conceivably alter mast cell response. Whether or not such clinical entities, such as systemic mastocytosis, might serve as a role model for such theories deserve additional study. The risk factors for individual idiosyncratic reactions (such as urticaria or bronchospasm) to opiates remain unknown, although pretreatment of subjects with mild intermittent asthma (≥20% decrease in FEV_1 after a standardized exercise test) with nalmefene (an opiate antagonist with 30 times the potency of naloxone) did not alter airway reactivity to exercise, suggesting that endogenous opioids probably do not play an important role in the pathogenesis of exercise-induced bronchospasm.[19]

Respiratory depression by morphine and similar pure mu opioid-receptor agonists, such as fentanyl and oxymorphine, probably produce their depressant effects through stimulation of mu receptors in the brain stem, producing strong inhibitory actions on respiratory parameters.[25] This effect, in addition to the direct histamine-releasing properties of opiates,[2] suggests a possible mechanism for the apparent work-related decreases in $PEFR_{max\text{-}min}$ observed in the workers of the present study. It is intriguing to speculate that the lack of significant reductions in $PEFR_{max\text{-}min}$ observed on Friday of the workweek may be due to mediator depletion from mast cells from repeated opiate exposure. The dramatic reductions in $PEFR_{max\text{-}min}$ observed on Monday, after a 3-day weekend with no exposure (and time for mediator accumulation), support this hypothesis.

Atopic status has not been studied with regard to opiate skin reactivity in humans; however, skin wheal-and-flare responses to morphine sulfate and histamine in Basenji greyhound dogs (considered to be a model of atopic disease) were found to have arisen from a significantly greater proportion of histamine released in response to morphine than in control dogs, suggesting greater responsiveness and greater releasability of mast cells in response to nonimmunologic stimulation.[20] In contrast to this observation, we did not find significant differences in skin test sensitivity or significant differences in wheal-and-flare response to histamine (data not presented) when the workers of the present investigation were stratified based on their atopic status.

Changes in the distribution of T-lymphocyte subpopulations in opiate addicts[21, 22] and, preliminarily, in the workers of the present study,[24] indicate the potential for alternative immunoregulatory mechanisms as an explanation for opiate hypersensitivity and diversity of mast cell activity.

This study suggests that human skin mast cell responses to the opiates are more widely disparate than was currently believed and that immediate dermal sensitivity may be used as a relative marker (compared to unexposed individuals) of opiate exposure in oc-

cupationally exposed individuals. In a previous work,[15] we reported no in vitro evidence for M-6-HS-HSA–specific IgE or IgG4 class antibodies in sera obtained from these groups of workers, although several of the workers complained of suspected opiate-related adverse health effects (tightness of the chest, skin rashes, and other symptoms) that are compatible with allergic (IgE) or short-term sensitizing IgG (IgG4)-mediated reactions. When the data of this study are combined with the lack of in vitro immunologic results, it appears that opiate hypersensitivity probably does not have an allergic etiology but most likely is associated with acquired pharmacologic hyperresponsiveness to the opiate compounds from exposure. In contrast to this finding, it is also possible that morhpine may be a weak allergen, and IgE production from exposure may occur in instances different from the occupational exposures reported in the present work. Specific IgE anibodies that react with morphine and codeine have been detected in the serum of a subject who experienced a life-threatening anaphylactic reaction after the administration of omnopon-scopolamine (papaveretum-hyoscine).[26] Hapten-inhibition studies with morphine and a number of structurally related analogues revealed that morphine and codeine were the most potent inhibitors of IgE binding to a morphine-solid phase. These data suggest that opiate exposure, at least in isolated instances, can cause the production of specific IgE.

We thank Charles Cooper of the Measurements Research Branch, Division of Physical Science and Engineering; NIOSH for performing mass spectroscopy analysis; Dr. S. Gupta and Mr. Jim Collins of the Hazard Evaluations and Technical Assistance Branch, Division of Surveillance, Hazard Evaluations and Field Studies, NIOSH, for performing the pulmonary function testing; Mr. Eric S. Baumgardner of Xavier University, Cincinnati, Ohio, for careful editing of the manuscript; and Miss Jocelyn M. Biagini for her capable assistance in the conduct of some of the statistical analyses of these data.

REFERENCES

1. Harrington JM. The health industry. In: McDonald JC, ed. Recent advances in occupational health. London: Butterworth, 1988:1-75.
2. Saucedo R, Erill S. Morphine-induced skin wheals: possible model for the study of histamine release. Clin Pharmacol Ther 1985;38:365-70.
3. Keffer JM, Bressler RB, Wright R, Kaliner MA, Metcalfe DD. Analysis of the wheal-and-flare reactions that follow the intradermal injections of histamine and morphine in adults with recurrent, unexplained anaphylaxis and systemic mastocytosis. J ALLERGY CLIN IMMUNOL 1989;83:595-601.
4. Fjellner B, Hagermark O. Potentiation of histamine-induced itch and flare responses in human skin by the enkephalin analogue, FK-33-824, beta-endorphin, and morphine. Arch Dermatol Res 1982;274:29-37.
5. Anderson K. Bronchospasm and intravenous street heroin. Lancet 1986;1:1208.
6. Oliver RM. Bronchospasm and heroin inhalation. Br Med J 1986;1:915.
7. Yahya MD, Watson RR. Minireview—immunomodulation by morphine and marijuana. Life Sci 1987;41:2503-10.
8. Donahoe RM, Buesos-Ramos C, Donahoe F, Madden JJ, Falek A. Mechanistic implications of the findings that opiates and other drugs of abuse moderate T cell surface receptors and antigenic markers. Ann NY Acad Sci 1987;496:711-21.
9. Romaguera C, Grimalt F. Occupational dermatitis from codeine. Contact Dermatitis 1983;9:170.
10. Alenina AN, Nikonets IF, Inova IS, Makarenko YL. Professional diseases of the skin and respiratory organs in persons working in the production of several drugs [translation]. Klin Med 1970;48:99-101.
11. Wainer BH, Fitch FW, Rothberg RM, Fried J. Morphine-3-succinyl-bovine serum albumin: an immunogenic hapten-protein conjugate. Science 1972;176:1143-5.
12. Wainer B, Fitch FW, Fried J, Rothberg RM. A measurement of the specific antibodies to morphine-6-succinyl-BSA by competitive inhibition of ^{14}C-morphine binding. J Immunol 1973;110:667-73.
13. Wainer BH, Fitch FW, Rothberg RM, Fried J. The structure of morphine monohemisuccinate. Science 1972;178:647-8.
14. Sawada J, Janejai N, Nagamatsu K, Terao T. Production and characterization of monoclonal antibodies against morphine. Mol Immunol 1988;25:937-43.
15. Biagini RE, Klincewicz SL, Henningsen GM, et al. Morphine antibodies in workers occupationally exposed to opiates at a narcotics-manufacturing facility. Life Sciences 1990;44:897-908.
16. American Thoracic Society. Standardization of spirometry—1987 update. Am Rev Respir Dis 1987;138:1285-96.
17. Privette PR, Shepherd MI. Narcotic allergy: true allergy or side effect. American Society of Hospital Pharmacists Midyear Clinical Meeting, 1988;23:164.
18. Roy BF, Bowen WD, Frazier JS, et al. Human anti-idiotypic antibody against opiate receptors. Ann Neurol 1988;24:57-63.
19. Christopher MA, Wyzan D, Harman E, Hendeles L. The effects of nalmefene, a potent oral antagonist, on exercise-induced bronchospasm. J ALLERGY CLIN IMMUNOL 1988;82:1037-41.
20. Turner CR, Darowski MJ, Sampson HA, Spannhake EW, Hirshman CA. Dermal mast cell releasability and end organ responsiveness in atopic and nonatopic dogs. J ALLERGY CLIN IMMUNOL 1989;83:643-8.
21. McDonough RJ, Madden JJ, Falek A, et al. Alteration of T and null lymphocyte frequencies in the peripheral blood of human opiate addicts: in vivo evidence for opiate receptor sites on T lymphocytes. J Immunol 1980;125:2539-43.
22. Donahue RM, Bueso-Ramos C, Donahoe F, et al. Mechanistic implications of the findings that opiates and other drugs of abuse moderate T cell surface receptors and antigenic markers. Ann NY Acad Sci 1987;496:711-20.
23. Casale TB, Bowman S, Kaliner M. Induction of human mast cell degranulation by opiates and endogenous opioid peptides: evidence of opiate and nonopiate receptor participation. J ALLERGY CLIN IMMUNOL 1984;73:775-81.
24. Henningsen GM, Biagini RE, Klincewicz SL, Gallagher JS, Trinkle LS. Flow cytometric analysis of peripheral leucocytes

from workers exposed occupationally to opiates. Toxicologist 1990;10:135.
25. Shook JE, Watkins WD, Camporesi EM. Differential roles of opioid receptors in respiration, respiratory disease, and opiate-induced respiratory depression. Am Rev Respir Dis 1990;142:895-909.
26. Harle DG, Baldo BA, Coroneos NJ, Fisher MM. Anaphylaxis following administration of papaveretum. Case report: implication of IgE antibodies that react with morphine and codeine and identification of an allergenic determinant. Anesthesiology 1989;71:489-94.
27. Klincewicz SL, Siwenski G, Fleeger A, Paluzzi L. Health-hazard evaluation (HHE) HETA 87-311, Penick Corp., Neward, N.J., U.S. Dept. of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, 1990:6.

Occupational asthma caused by α-amylase inhalation: Clinical and immunologic findings and bronchial response patterns

Eloy Losada, PhD, Miguel Hinojosa, MD, Santiago Quirce, MD, Moisés Sánchez-Cano, MD, and Ignacio Moneo, MD *Madrid, Spain*

Inhalation of dust from different enzymes can be the cause of occupational asthma in exposed workers. α-Amylase, derived from Aspergillus oryzae, *is one of these enzymes, although there are few studies in the medical literature that refer to its allergologic properties and to clinical studies in sensitized patients. The results obtained in a study performed in 83 pharmaceutical-industry workers exposed to powdered α-amylase are described in this article. The existence of sensitization to this enzyme was demonstrated in 26 of the workers by positive skin tests. Specific IgE values were significantly higher in workers with positive skin tests than in workers with negative skin tests ($p < 0.001$). The bronchial provocation test with α-amylase was positive in six of the 14 patients challenged, and only immediate bronchial responses were observed; the same type of response was obtained by nasal provocation. One of the workers had a positive response to oral provocation with this enzyme, presenting abdominal, skin, and respiratory symptoms a few minutes after ingestion. Consequently, we consider that the bronchial asthma presented by the workers was due to an immediate-type, IgE-dependent, immunologic mechanism. (J ALLERGY CLIN IMMUNOL 1992;89:118-25.)*

Key words: *α-Amylase,* Aspergillus oryzae, *enzymes, bronchial provocation test, occupational asthma*

Some enzymes, independently of their origin, are of increasing importance in allergic pathology, particularly in the working environment in which they are often the etiologic agents of occupational asthma.

Various authors have described cases of rhinitis and BA of allergic mechanism in workers exposed to the dust of diverse enzymes, demonstrating their great sensitizing capacity.[1-9]

In the last few years, interest has been demonstrated in α-amylase, a glycolytic enzyme with a molecular weight of 51,000 daltons. It can be obtained from different sources, such as human saliva, pig pancreas, *Bacillus subtilis, Aspergillus oryzae,* etc.

Abbreviations used
BA: Bronchial asthma
PBS: Phosphate-buffered saline
REIA: Reverse-enzyme immunoassay
OD: Optical density
RT: Room temperature

From the Departments of Allergy and Immunology, Hospital Ramón y Cajal, Madrid, Spain.
Received for publication Jan. 16, 1991.
Revised Aug. 6, 1991.
Accepted for publication Aug. 26, 1991.
Reprint requests: Eloy Losada Cosmes, MD, Servicio de Alergia, Hospital Ramón y Cajal, Ctra, Colmenar Km 9,1, 28034 Madrid, Spain.
1/1/33370