

Nightly asthma caused by allergens in silk-filled bed quilts: Clinical and immunologic studies

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*Bed quilts filled with silk waste were found to cause nightly attacks of asthma. The first symptoms appeared on an average after 7 mo of exposure at a mean patient age of 30 yr. About 50% of the patients could be classified as atopic. Extract of the filling material revealed positive scratch and RAST tests in most patients. The bed quilts were advertised as being filled with pure wild silk (from silk moths of the genus *Antheraea* feeding on oak leaves), but it was found that in most cases the filling also included waste of cultivated silk (*Bombyx mori*). The origin of the allergen was sought. Since textile products of silk are considered to be practically nonallergenic, some type of contaminant appeared the most likely candidate. No allergen was found in cocoons, chrysalis (pupa), or moths of *Antheraea*. However, some cocoons of *Bombyx mori*, usually in small amounts, and excretions of the silkworm did contain allergen. High concentrations of allergen were found in an extract of living insects of the genus *Anthrenus* that was present in one batch of *Bombyx mori* cocoons. Thus, the silk waste appeared to be contaminated with material from the silkworms and also with infesting insects, which together create a very potent allergen repertoire that after a short incubation time, in a considerable frequency, elicits a rise in asthmatic symptoms and high serum IgE antibody levels. (J ALLERGY CLIN IMMUNOL 75:452-9, 1985.)*

According to Chinese legend, silk culture dates back to the year 2640 BC, when the Empress Si-Ling-Chi learned how to rear the silkworms and unwind the cocoons that they made. For 3000 yr China held a monopoly in the silk industry. Sericulture, the silk-production industry, then spread to Japan and later westward over Asia. China and Japan still dominate the world production; of the total 52,300 tons of raw silk produced in 1981, their shares were 22,400 and 14,800 tons, respectively.¹ Large quantities are also produced in India and the Union of Soviet Socialist Republics, but no significant amounts are produced in Western Europe or the United States.

Allergy to silk products is well-known as an oc-

cupational hazard in sericulture.^{2,3} Asthma and rhinoconjunctivitis occur among workers involved in several stages of the production. Potent allergens have been isolated from excretory products from the ripe larva and from scales from the moth, the chrysalis, and the cocoon.⁴ In contrast, textile products of silk have rarely been incriminated as a cause of allergic reactions.

In the late 70s bed quilts filled with silk were introduced on the market in Switzerland, Germany, and Austria. They rapidly became popular. By the end of 1980, approximately 10,000 bed quilts of one brand only had been sold in Switzerland alone, and today between five and 10 different brands are available.

In 1979 some patients were found to be having nightly attacks of allergic asthma that proved to be associated with bed quilts of a particular brand (Mandarin; Brinkhaus Ag, Warendorf, West Germany)⁵; later, bed quilts of other brands (Dorseide and Dorbena [Dorbena Ag, Schaan, Switzerland], Patricia, and Billerbeck) were also linked to the same symptoms. Since that time, more than 50 such patients have been investigated at the allergy clinic in Zürich,⁶ and some patients have also been discovered in Germany and Austria.

The bed quilts eliciting an allergic reaction were,

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according to the manufacturer's declaration of contents, stuffed with silk from wild silk (wildseide) moths living from oak leaves. This article reports some clinical data on patients with asthma caused by the bed quilts and also describes studies on the immunologic and physicochemical properties of the allergens associated with the silk waste used to fill the bed quilts. It was found that the allergens were associated with waste of mulberry silk (*Bombyx mori*) rather than with wild silk (*Antheraea* spp.), but none of the classic silk allergens appeared to have been responsible. In addition, material of the insect *Anthrenus* contained allergens that were present as a contamination of the silk waste.

MATERIAL AND METHODS

Study subjects

From the beginning of 1979 to the end of 1982, a total of 50 patients were identified at the Department of Dermatology and Allergy of the University Hospital, Zürich, Switzerland, as having an allergic asthma caused by bed quilts filled with silk waste. Three of these patients worked in the silk industry. The diagnosis was based on the case history, prick and intradermal skin tests, RAST, and serum IgE analyses.⁵ An individual was classified as atopic when he had a family and case history of classic atopic allergy (extrinsic asthma, rhinoconjunctivitis and atopic dermatitis) combined with several positive skin tests and sometimes increased IgE levels.

Skin scratch tests were performed with intact filling from a Mandarin bed quilt moistened by saline that was put on a fresh scratch in the skin. The results were graded from 0 to 4+ in relation to a negative (saline) and a positive control (histamine hydrochloride, 0.1 ml/ml). A commercial silk extract (*Bombyx mori*) (Hollister-Stier, Seattle, Wash.) was used in intradermal skin tests at 1/1000 w/v.

Venous blood was obtained from the patients, and the serum samples were stored frozen. A large number of serum samples from patients with other types of atopic allergy referred to the Department of Clinical Immunology, Karolinska Hospital, Stockholm, for RAST-based routine allergy diagnosis as well as from healthy blood donors were also available. A serum pool to which myeloma IgE had been added to reach an IgE concentration of approximately 4000 kilo units per liter (kU/L) was used as a high IgE control for nonspecific background binding to the discs in RAST.

Allergen material

Filling material for bed quilts of various brands (Mandarin, Patricia, Dorbena, and Filt 500/82) were obtained through the president of the International Silk Association, Mr. H. Weisbrod, Weisbrod-Zürcher AG, Hausen a.A., Switzerland. According to the declaration of contents, the material consisted of pure wild silk (wildseide). This silk is obtained from oak silk moths of the *Antheraea* spp. However, microscopy studies (performed by Testex AG, Zürich,

TABLE I. A summary of the clinical characteristics of the first 50 patients with asthma caused by bed quilts filled with silk waste

	No. of patients	% Of patients
Average age at first manifestation (yr) 30 (10 to 58)		
Interval between exposure and symptoms (mo) 7 (1 to 36)		
Ratio M:F	23:27	
Main symptom		
Asthma	50	100
Rhinitis	24	48
Conjunctivitis	15	30
Preexisting inhalation allergy	12	24
Atopy in screening tests	29	59
Monovalent sensitization to silk	21	41
IgE level over 200 U/ml	23	47
IgE level increased in monovalent silk sensitization	8	16
Success of exposure prophylaxis	49*	98

*Seven patients with preexisting asthma demonstrated considerable improvement.

Switzerland) revealed that the silk quality should be classified rather as waste and that in all cases but one (Dorbena) the silk waste was a mixture of wild silk and cultivated silk (*Bombyx mori*).

Silk waste preparations of different kinds (kheba silk and tussah silk), supposedly of the same quality as that found in the bed quilts, were also supplied by Mr. H. Weisbrod.

A preparation of tussah silk fibers imported from Germany by a Swedish textile company for use as a minor (15%) constituent of yarn for heavy material (for furniture and draperies) was also tested.

Sericin, a glue-like substance cementing the two fibroin filaments of the silk into a single thread, was obtained in a purified form from Prof. Dr. med. habil. E. Fuchs, Wiesbaden, West Germany. This preparation from *Bombyx mori* was used by Dr. Fuchs in an early study of silk allergy.³

Cocoons with living chrysalises of *Antheraea pernyi* were kindly supplied by Professor Hans G. Boman, Department of Microbiology, University of Stockholm, Sweden. The cocoons were hatched in the Department of Clinical Immunology, Karolinska Hospital, Stockholm. Studies of cocoons, chrysalises, and hemolymph of chrysalises and moths were performed.

Cocoons, chrysalises, and silk waste of various other kinds of wild silk (tasar/tussah, muga, and eri) as well as soil from several sericulture centers in India were kindly

TABLE II. Examples of results of RAST to wild silk waste compared with skin scratch tests by use of a 1/10 w/v extract of bed cover filling

	RAST		Skin test	IgE kU/L	Atopy
	Class	PRU/ml			
Patient					
U. S.	4	327.0	4+	1280	+
Z. G.	4	73.0	3+	525	+
L. C.	4	52.0	3+	510	+
F. E.	4	49.0	3+	180	—
H. E.	4	21.0	3+	475	—
H. C.	4	18.0	3+	170	+
W. M.	3	9.7	3+	185	—
S. T.	3	8.5	2+	745	—
Controls*					
S. J.	0	<0.2	NT†	NT	—
S. M.	0	<0.2	NT	NT	—
W. R.	0	<0.2	—	NT	+
D. H.	0	<0.2	—	55	+

Atopy was defined on the basis of the case history and skin tests against common allergens. The results of the eight most strongly RAST-positive patients are illustrated.

*Four patients with other allergies than silk waste are presented as control subjects.

†NT = not tested.

provided by Mr. H. Weisbrod. In addition, he supplied us on several occasions with cocoons, chrysalises, and live moths of cultivated silk, *Bombyx mori*.

Some cocoons of *Bombyx mori*, after storage in closed boxes at room temperature for some months, were found to be heavily infested with an insect identified (kindly performed by A. Wiren, Ph.D., University of Uppsala, Uppsala, Sweden) as belonging to the genus *Anthrenus*. Since this or any other insect has never been detected in this laboratory, it is likely that it represents a contamination of the cocoons. The mature insects and larvae were collected manually and extracted. Extracts were also prepared from *Chironomus* spp., *Cladotanytarsus lewisi* (green nimitti) (kindly supplied by Dr. R. Tee, Brompton Hospital, London), and *Aedes communis*.

RAST reagents

Extracts, usually 1/10 w/v, in phosphate-buffered saline of neutral pH or chromatography fractions were coupled to CNBr-activated filter paper discs essentially as described in detail for CNBr-activated microcrystalline cellulose⁷ for determination of either IgE antibody in serum or allergen in solution. Each batch of discs coated with an allergen extract was tested in RAST against a pool of sera from patients allergic to silk waste for their IgE antibody binding capacity, against a high IgE control serum, and a serum from a healthy individual with a normal IgE level for determination of nonspecific trapping of IgE. Papain (mercuripapain, 22 mg/ml, Sigma Chemical Co., St. Louis, Mo.) was coupled to CNBr-activated discs (1 to 10 μ l to 20 discs). The discs had a high IgE background, binding 11% to 13% of added tracer when these discs were tested

against the high IgE control serum. The background was normal for low and normal IgE levels.

Commercially available discs with the following allergens were used: oak pollen, house dust, *Dermatophagoides pteronyssinus*, chymopapain, cockroach, cat dander (Phadebas RAST, Pharmacia Diagnostics, Uppsala, Sweden), and pollen of *Morus alba*, castor bean, rat and mouse urine proteins, and storage mites (*Lepidoglyphus destructor*, *Acarus siro*, and *Tyrophagus putrescentiae*) (Allergo-Discs, MIAB, Knivsta, Uppsala, Sweden).

IgE antibody determination

The allergen-coated discs were used in RAST⁸ to measure IgE antibodies in serum. One allergen disc was incubated with 50 μ l of undiluted serum or, when it was necessary because of a high IgE antibody concentration, diluted in a RAST diluent consisting of a pool of normal, nonallergic sera with low (<20 kU/L) total IgE levels and with no IgE antibody detectable by RAST to any common allergen. After 3 hr of incubation at room temperature, the discs were washed three times by the addition of approximately 2.5 ml of saline containing 0.1% Tween 20 followed by aspiration. The discs were then aspirated dry, and 100 μ l of ¹²⁵I-labeled anti-IgE of De2 specificity,⁹ corresponding to 50,000 to 70,000 cpm, were added to each tube for an overnight incubation. The discs were then washed again as described above, and their count rates were determined in a gamma scintillation counter (Rackgamma or Multigamma; Wallac, LKB, Bromma, Sweden). The results were expressed in Phadebas RAST unit per milliliter as derived from the Phadebas RAST Reference System (Pharmacia Diagnostics AB, Uppsala, Sweden).

Allergen determination

Allergen in extracts and chromatography fractions was also determined by RAST inhibition.¹⁰ Equal volumes of an allergen solution and a pool of sera positive to silk in RAST diluted in RAST buffer (0.05M phosphate, pH 7.5, 0.15M NaCl, 0.2% bovine serum albumin, 0.1% Tween 20, and 0.02% NaN₃) to a count rate on silk discs of approximately 10,000 cpm/50 µl were mixed and incubated overnight at room temperature. Serial tenfold dilutions of a reference silk extract (kheba silk, 1/10 w/v) with a concentration of 100 arbitrary units, were included in each experiment. Fifty microliters of the mixture in duplicate were transferred to empty test tubes. Silk allergen discs were added, and a RAST was performed as described above. The results were expressed either in relation to the reference extract or in percent of a buffer control representing 100% binding.

Allergen extracts were fractionated by gel filtration on Sephadex G-100 in 0.05M phosphate buffer, pH 7.5, containing 0.15M NaCl and 0.02% NaN₃, by use of a glass column (K 26/100) 25 mm wide and 1000 mm high, with a total bed volume of 446 ml (Pharmacia Fine Chemicals AB). The flow rate was 16.7 ml per hour. Fractions of 2.5 ml were collected and analyzed for their allergen content by RAST. Separations were also performed by ion exchange chromatography on an FPLC system (Pharmacia Fine Chemicals AB) by use of a Mono-Q-column. The starting buffer was 0.025M phosphate, pH 6.5. The gradient used was 1.0M phosphate buffer, pH 6.5.

For determination of the protein concentration of the allergen extracts, folin reagents¹¹ were used with bovine serum albumin as standard.

RESULTS

Clinical aspects

During a 3-yr period, 50 patients were diagnosed as having nightly asthma caused by allergy to bed quilts filled with silk waste. On an average, the first symptoms appeared after 7 mo of exposure, a remarkably short time, especially considering that only 24% had had a previous inhalation allergy and that 59% could be regarded as atopic from the results of the case history and skin testing (Table I). Thus, 21 patients (41%) had a monovalent allergy to silk waste. Eight of the 23 patients with an IgE level above 100 kU/L were exclusively allergic to silk.

Many of the patients had very high RAST class 4 levels of IgE antibodies to silk waste as measured by RAST (Table II). Of the six patients with RAST class 4 levels of IgE antibodies to silk, two were classified as not being atopics.

Immunologic studies

Extracts of silk waste found in various bed quilts and coupled to discs revealed positive results in RAST when extracts were tested against sera from patients with allergy to the bed quilts. The concentrations of

TABLE III. Silk waste allergen contents in various silk materials expressed in arbitrary units per milligrams of protein and per milliliters in a 1/10 w/v extract

Allergen source	Silk allergen	
	U/mg	U/ml
Mandarin	123	74.0
Kheba silk	28	100.0
Dorbena*	21	3.9
Patricia	13	4.8
<i>Bombyx mori</i> , cocoons†	23	9.5
Filt 500/82	3	2.3
Tussah silk‡	2	0.4
Tussah silk	1	0.4
<i>Bombyx mori</i> , cocoons§	0.5 to 0.7	1.4 to 1.9
Secretion	0.600	3.600
Cocoon wash	0.400	0.400
Cocoons†	0.600	0.200
Butterfly	0.020	0.100
Sericin	0.003	0.005
<i>Antheraea</i>	0.001	0.005

A preparation of kheba silk was used as reference and was assigned a value of 100 arbitrary units per milliliter (for details, see text).

*Does not contain *Bombyx mori* fibers.

†These cocoons of *Bombyx mori* were obtained from Mr. H. Weisbrod, Weisbrod-Zürcher Ltd., Hausen, Switzerland, on two occasions about 6 months apart.

‡A preparation of raw silk coils of tussah silk imported to Sweden and used to prepare a yarn.

§Also from Mr. H. Weisbrod but on a third occasion. The silk moths were hatched in our laboratory at Karolinska Hospital, Stockholm.

||The preparation was kindly supplied by Dr. E. Fuchs, Wiesbaden, West Germany.

allergen in the extracts were determined by RAST inhibition by use of a preparation of kheba silk as reference and expressed in arbitrary units per milliliter per milligram of protein (Table III). The highest content, 123 U/mg, was found in the filling of the Mandarin bed quilt, which was the first brand of bed covers noticed to cause allergy.

In an attempt to trace the origin of the silk material containing the allergen, various types of wild silk wastes available in bulk were tested. The preparation of kheba silk was found to have a very high concentration of allergen, but high concentrations were also found in a preparation of tussah silk (Table III). A preparation of wild silk imported to Sweden for inclusion in a yarn consisting of wild silk and synthetic fibers was also tested and found to contain allergen. However, no allergen could be detected in an extract from the yarn that contained 15% wild silk.

Cocoons, chrysalises (pupa), and moths of wild silk

TABLE IV. Results of RAST (PRU/ml) to various silk-related allergen preparations

Patient serum	Silk waste	Silkworm* excretions	Silkworm† excretions	Dust‡	Anthrenus
U. S.	144.0	0.7	2.1	3.8	8.3
Z. G.	94.0	0.4	1.4	1.4	1.3
F. E.	49.0	0.6	2.3	2.3	2.6
W. M.	9.7	0.3	0.3	1.2	2.4
K. E.	8.8	0.6	2.9	2.0	5.7
R. M.	7.0	0.3	0.8	0.9	2.3
M. K.§	14.0	20.0	0.3	15.0	6.0

*This ripe larva (*Bombyx mori*) urine antigen preparation, the dust sample, and serum of M. K. were kindly provided by Professor S. Kobayashi and Dr. T. Nakazawa, Gunma, Japan.

†Liquid silkworm (*Bombyx mori*) excrements kindly provided by Mr. Weisbrod, Zürich. The larvae had been reared under experimental conditions in Switzerland.

‡Dust collected at the sericulture work place. It may contain products of *Bombyx mori*, dry leaves of the mulberry tree, and anything else to be found on the floor.

§This patient, suffering from occupational asthma, is working in the sericulture industry.

(*Antheraea pernyi*) were extracted, and the extract was coupled to discs for RAST testing. No positive RAST results were obtained. The studies were extended to include other species of wild silk (tasar/tussah, muga, and eri), but these elicited no positive RAST results.

The possibility that the bed quilt filling might contain allergen from the mulberry silk moth (*Bombyx mori*) was explored. Several batches of cocoons, chrysalises, and moths were investigated during a period of several months. In one batch of cocoons, high concentrations, 23 U/mg, were found, whereas in others little (0.7 to 0.06 U/ml) or no allergen was detected (Table III). No allergen was found in extracts of the moths or chrysalises. One preparation of excretory products from ripe larvae (kindly supplied by Professor S. Kobayashi, Gunma, Japan) contained very little allergen according to our test system (0.005 U/mg) despite a strong reaction with a serum (M. K.) of a Japanese sericulturist with silk asthma (Table IV). Another silkworm excretion preparation (kindly supplied by Mr. Weisbrod) elicited moderately strong reactions with our serum samples, but no reaction with serum of M. K. (Table IV). Purified sericin, dissolved in saline or alcohol or sham-treated by heating in 0.2% Na₂CO₃ and 0.3% Tween 20 to simulate the reeling process, was not allergenic either when coupled to discs or in inhibition experiments.

The possibility that the allergen was derived from a solid contaminant of the silk waste was examined. The kheba silk preparation appeared on inspection to be heterogeneous, consisting of white and grey threads and some dark colored small grains of uneven sizes. The threads were separated manually from a small batch of the kheba silk waste. Extracts, 1/10 w/v, of the separated threads and also of what was left were prepared. The thread extract was noncolored and contained high concentrations of allergen, the positive

serum pool revealed a RAST value of 10.1 PRU/ml, and all controls were negative. The same value for the other slightly dark colored extract was 1.1 PRU/ml. The relative silk allergen contents were 100 and 4.3 U/mg, respectively. These results indicate that the allergen has affinity for the silk threads.

In the search for possible external contaminants, the silk RAST positive sera were analyzed by RAST against several allergens such as oak and mulberry tree pollen, castor bean, house dust, rat and mouse urine proteins, cat dander, cockroach, insects of the genus *Chironomus*, *Cladotanytarsus lewisi*, *Aedes communis*, house dust mites, and three species of storage mites. No positive RASTs were obtained. A serum strongly RAST positive to the two-spotted spider mite¹² (kindly provided by Dr. F. Björkstén, Helsinki, Finland) was negative to silk waste.

Discs coated with the extract of *Anthrenus* elicited moderately strong reactions in RAST with the silk RAST positive sera (Table IV). However, in no case was the reaction as strong as that of the silk waste.

Separation of allergen activity

The kheba silk extract was separated by cation exchange chromatography on an FPLC Mono-Q-column (Pharmacia Fine Chemicals AB) (Fig. 1). Each fraction was coupled to CNBr-activated discs and tested with RAST against the silk waste positive serum pool. A confluent double peak of RAST activity with a broad base was observed, indicating heterogeneity of the allergenic material.

A 4 ml sample of an extract of silk waste from the Mandarin bed quilt (extract 1/10 w/v concentrated 20 times by lyophilization) was separated by gel filtration on Sephadex G-100 (Fig. 2). Every third fraction was tested for its allergen content by RAST inhibition. Again, a heterogeneous peak with a very broad base

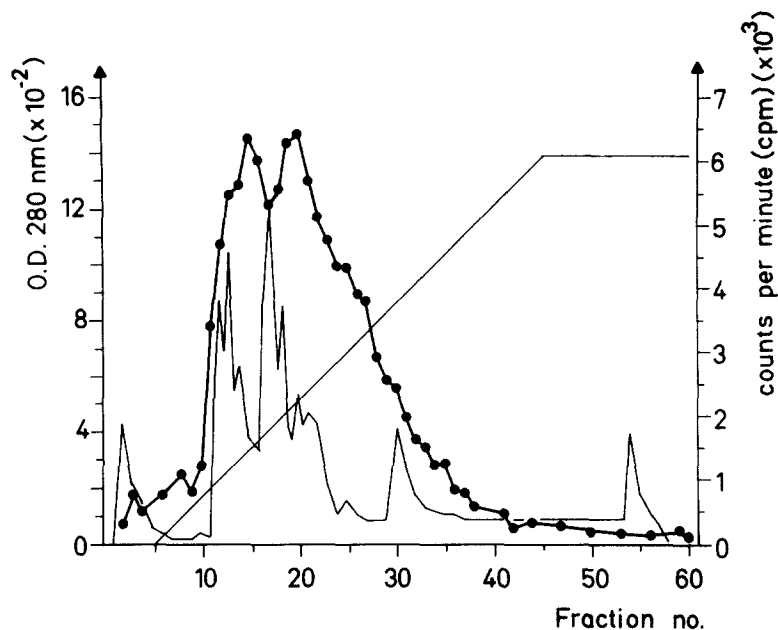


FIG. 1. Separation of a Kheba silk extract by cation exchange chromatography on an FPLC Mono-Q-column (Pharmacia Fine Chemicals AB). Silk allergen (●—●) was measured by RAST inhibition. The straight line represents the gradient, 0.025 – 1.0M phosphate, pH 6.5.

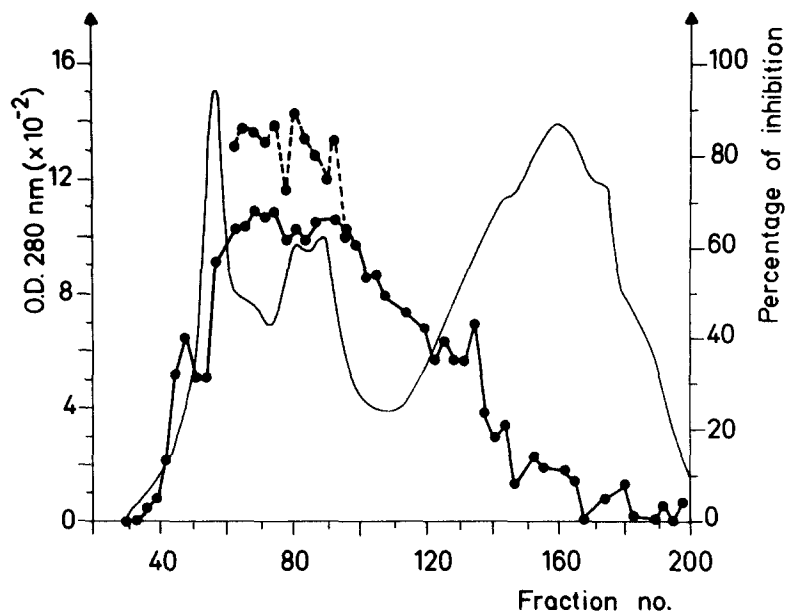


FIG. 2. Gel filtration on Sephadex G100 of an extract of silk waste from a bed quilt. Silk allergen was measured by RAST inhibition by use of fractions undiluted (●—●) or 1/10 diluted (●---●).

was obtained. Maximum allergen activity was found to elute in fractions corresponding to a molecular size of approximately 60,000 dalton.

DISCUSSION

Allergy to silk products is well-known as an occupational hazard in sericulture. Although the principal source of silk for production is derived from

Bombyx mori, the domesticated species of silkworm feeding on the leaves of the mulberry tree, there is a considerable trade in some countries in silk from silkworms living "wild." The most important type, known as tussah silk, is a product of several species of the genus *Antheraea*, most of which feed on oak leaves.

In sericulture the workers are exposed to potentially

allergenic substances derived from the various stages of the silkworm in its transition from egg to butterfly or moth. Important allergens are found in excretory products of the silkworm, hemolymph of the chrysalis (pupa), dust from the moth, and sericin, a gluey substance from the cocoons.⁴

The silk thread, which consists of two filaments glued together with sericin, is reeled off the cocoon. From an undamaged cocoon a single unbroken filament as much as a mile long can be collected. Slightly broken filaments are used to make spun silk. Severely broken threads and damaged cocoons, that is the silk waste, cannot be used for spinning but have recently found a use as a filling material of bed quilts.

The silk waste used to fill bed quilts of certain brands obviously contains some very potent allergens capable of producing an IgE-mediated respiratory allergy in persons using these bed quilts regularly. The potency of the allergen is illustrated by the surprisingly high ages of some of the patients, the short sensitization time, and the very high IgE antibody concentrations in the serum of some of the patients.

The producers of these bed quilts initially advertised their products as containing allergen-free wild silk. Thus, it was believed that the allergen was derived from silk moths feeding on oak leaves (*Antheraea* spp). However, no allergen was detected in cocoons, chrysalises, or moths of these species. The studies were then extended to include material of mulberry silk, spun by *Bombyx mori*. Allergens were now found in some but not all of the cocoons studied. However, no allergen was found in material from the chrysalises or moths, which are classic sources of allergen in the sericulture industry. That in most cases the allergen was associated with *Bombyx mori* material was further supported by microscopic investigations of the silk waste, which revealed silk fibers of *Bombyx mori* origin. However, in one bed quilt associated with allergic asthma, no *Bombyx mori* fibers could be found.

The variations in allergen concentration of various batches of *Bombyx mori* cocoons were in the order of 10,000-fold. The highest concentrations of allergen were found in the various kinds of silk waste preparations studied. These were obtained from bed quilts of different producers, but silk waste preparations intended for other uses also contained allergen. Thus, one preparation of silk waste, claimed to be wild silk, was sold by a German company to a Swedish spinning mill. The material was mixed with synthetic fibers to make a yarn for production of heavy material for curtains and furniture. This wild silk preparation contained quite large amounts of allergen, but no allergen was found in the yarn. This does not rule out the

possibility that enough allergen to cause sensitization may be present in fabrics made of this yarn.

Some allergen activity was found in the preparation of partially purified excretory product antigens of the *Bombyx mori* silkworms obtained from Japan. Thus, contaminating protein antigens from the silkworm are responsible for some of the allergenic activity of the silk waste. However, a similar but more crude preparation from larvae reared under experimental conditions in Switzerland was negative in our tests.

In the reeling process, the unwinding of the fine silk filaments from the cocoons is started by soaking the cocoons in hot water to soften the sericin gum that holds the filaments together. Sometimes proteolytic enzymes such as papain are used to loosen the fibers. It is thus a possibility that sericin or papain is left on the silk thread. Sericin has been claimed previously to be a potent silk allergen.³ However, we did not find that sericin could act as an allergen by directly binding IgE antibody or by inhibiting the reaction between IgE antibody and the unknown allergens in the silk waste. In order to mimic the reeling process, sericin alone or together with human serum albumin was heated in an alkaline buffer containing detergent. This procedure did not lead to the appearance of allergenicity. Similar handling of an active allergen extract led to a decrease in allergen potency. RAST was also performed with papain discs, but no positive result was obtained.

The most likely explanation for the wide variations in allergen concentration and also for the finding that in almost every case the silk waste contained higher concentrations of allergen than any *Bombyx mori* cocoons is that the allergens represent some kind of external contamination. The allergenic contaminants must have some affinity for the silk fibers, since an extract made of manually selected silk fibers from the silk waste contained more than 10 times as much allergen as an extract of the rest of the material, probably mostly vegetable fibers. The material must have been contaminated in close connection with the sericulture centers since both cocoons and silk waste contain the allergens. However, since treatment of silk waste mimicking the reeling process led to loss of allergenicity, the material was probably contaminated after the reeling process, most likely during storage.

Several types of possible external contaminants were studied. Castor bean contains a very potent allergen that often contaminates burlap sacks in which the beans are stored.¹³ Since *Antheraea* spp. feed on oak leaves and *Bombyx mori* are mulberry feeders, it is possible that the waste might contain such pollens or leaves. Preparations of European house dust and extracts of soil from sericulture centers in India as

well as house dust mites, three kinds of storage mites, and various mosquitoes and cockroaches were also tested. Rat and mouse urine proteins were used as an example of possible contamination of dust by animal protein. All the allergens mentioned were tested by RAST with negative results.

The moderately strongly positive RAST results for the extract of *Anthrenus* are very interesting. Larvae of this genus are known to feed on dry caterpillars, butterflies, and other insects, and they are a considerable problem in museums and institutes of entomology in which they can destroy insect collections. Protein material of *Anthrenus* is obviously one important allergen in the silk waste.

Silk waste used to fill bed quilts contains several very potent allergens, some of which are associated with *Bombyx mori* silk, whereas others are external contaminants like the material of *Anthrenus*. However, an extract of the bed quilt content elicits a stronger reaction in RAST than can be accounted for by the allergens identified so far. Obviously, not only one but several allergens are involved, a finding supported by the results obtained in gel filtration and ion exchange chromatography separation experiments. It is likely that more allergens are to be found among other insect material contaminating the silk waste. The insects studied in this work do not demonstrate any significant degree of allergenic cross-reactions. It is likely that insect material in dust as a source of inhalation allergens is of greater clinical significance than is appreciated today.

Allergy to silk waste used in yarn for production of heavy fabrics and as filling material for bed quilts is becoming an increasing problem in medical care. Better methods should be used for quality control of the silk waste used to stop this hazardous trend.

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