

INSECTS AS INHALANT ALLERGENS

CONSIDERATION OF AEROBIOLOGY, BIOCHEMISTRY, PREPARATION OF MATERIAL, AND CLINICAL OBSERVATIONS

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"How many insects are there? And how many kinds of insects? Maybe we shall never know. But wherever we go and whether we see them or not, we are surrounded by countless millions of insects. Every forest, every field, every back yard, every roadway is a gigantic insect zoo. A wide world of endless variety and interest is open to all who will do a little investigating on their own."

—Curtis W. Sabrosky.¹

INTRODUCTION

SINCE common usage has caused the word *insect* to include most of the arthropods, this terminology will be used throughout the present discussion. This will also conform with the broad title of the Insect Allergen Subcommittee of the Research Council of The American Academy of Allergy, which has encouraged many of these studies. When the class of true insects is singled out, the term *Hexapoda* will often be used.

The role of insects in allergy has been recorded, for the most part, as a series of isolated case histories in which the allergy was attributed to a variety of arthropods, chiefly of the class *Hexapoda*. The list of insects as inhalant allergens is constantly lengthening and is summarized in Table I. Reactions to biting and stinging insects are excluded from this discussion.

It would seem important, therefore, that the entire insect world be better evaluated—not only in its distribution and relationship to clinical symptoms but even further as concerns the nature of the allergenic properties. Most recently, Feinberg and colleagues²⁸ have aroused intense awareness of the broad nature of this problem. The Insect Allergen Subcommittee is planning to accumulate clinical experiences, biochemical analyses, immunologic studies, and entomologic data to provide a link between the obvious antigenicity of the insect allergen and clinical disorders. Coordinated efforts might eventually provide a more direct approach in understanding and treating many obscure and discouraging allergic problems.

RELATIONSHIP OF INSECTS TO ALLERGIC DISORDERS

If the postulates of Thommen²⁹ for pollinating plants causing hay fever are applied to insect allergens, much convincing evidence is revealed by paraphrasing these simple but basic postulates:

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1. The insect must contain an excitant of allergenic symptoms.
2. The insect must appear in sufficiently large numbers.
3. The insect must be widely and abundantly distributed.
4. The insect must be anemophilous, or wind borne, during some stage of its life.
5. The insect must be sufficiently buoyant to be carried considerable distance.

First Postulate.—Evidence that insects contain excitants of allergic symptoms is brought out by numerous specifically positive direct skin reactions and by passive transfer reactions as well (Figs. 1 and 2). There are also many convincing and confirmed reports of clinical symptoms produced by most of the major orders of the class *Hexapoda* and of clinical benefit from treatment with the specific insect allergen. The exact nature of the excitant (or excitants) is not entirely clear, and more detailed studies on the biochemical structure and immunologic activities will be elaborated upon later in this discussion.

TABLE I. ARTHROPODS INCLUDED IN OUR STUDIES

<i>Class—Arachnida</i>	<i>Class—Hexapoda (Cont'd)</i>
Araneae (spiders)	<i>Order: Coleoptera</i>
*Acarina ² (mites, ticks)	*Beetles ^{11, 12}
	*Bean weevil ¹³
<i>Class—Crustacea</i>	<i>Order: Neuroptera</i>
Sowbugs	Lacewing flies
*Daphnia (water flea) (3)	<i>Order: Trichoptera</i>
Shrimp (plankton)	*Caddis flies ¹⁴⁻¹⁷
Shrimp (edible)	<i>Order: Lepidoptera</i>
Crab	*Moths, butterflies ¹⁸⁻²¹
<i>Class—Hexapoda (Insecta)</i>	<i>Order: Diptera</i>
<i>Order: Orthoptera</i>	*Houseflies ²²
*Locusts ^{4, 5}	*Mushroom flies ^{23, 24}
Grasshoppers	*Sewer flies ²⁵
Cockroaches	*Midges ²⁶
*Crickets ⁶	Deer flies
<i>Order: Isoptera</i>	Black flies
Termites	<i>Order: Siphonaptera</i>
<i>Order: Dermaptera</i>	Rat fleas
Earwigs	<i>Order: Hymenoptera</i>
<i>Order: Ephemeroptera</i>	*Honeybees ²⁷
*May flies ^{7, 8}	Bumblebees
<i>Order: Hemiptera</i>	Hornets
*Bedbugs ⁹	Wasps
<i>Order: Homoptera</i>	Parasitic wasps
*Aphids ¹⁰	Yellow jackets
	Ants

Asterisk (*) denotes those arthropods reported by others as causes of allergic rhinitis and asthma.

Second Postulate.—The appearance of insects in large numbers is evident even to the casual observer. This may be a seasonal variant not only peculiar to the climate but also predictable for certain specific insects. The caddis flies

Fig. 1.

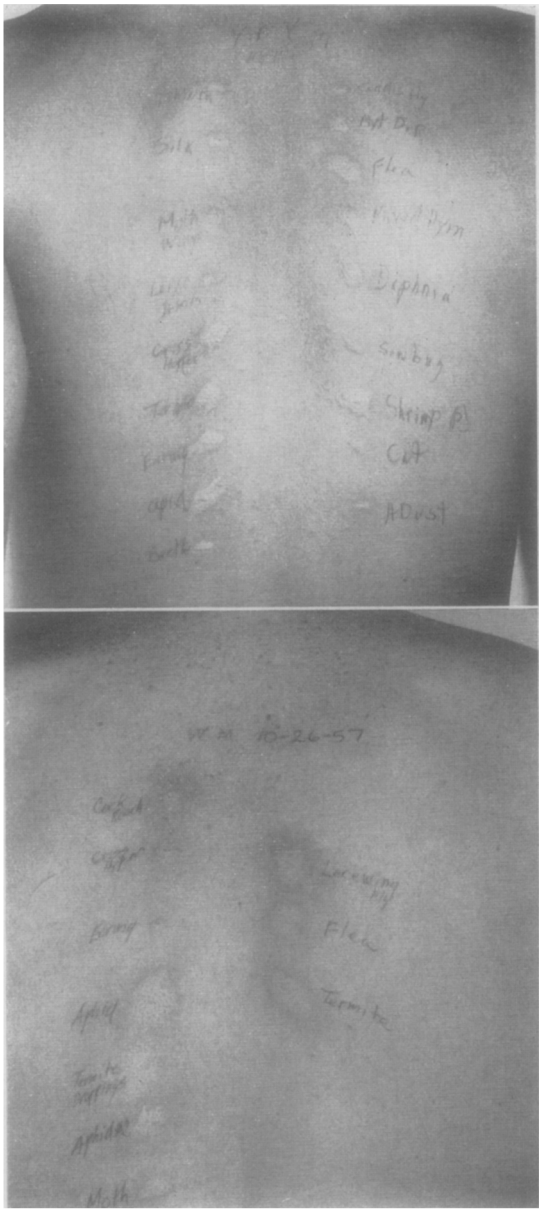


Fig. 2.

Fig. 1.—Multiple reactions by scratch method to *Hexapoda* and *Crustacea*. Note lesser reactions to cat epidermal and house dust.

Fig. 2.—Varying reactions to several hexapods. The strongest reactions are to two samples of aphids.

and May flies exemplify this very well. In temperate climates in both the late spring and the waning summer and autumn months the appearance of many such insects in what might be considered "clinical numbers" can be predicted. From Biblical times to the present age of insecticides, swarming clouds of insects have plagued us.

Third Postulate.—The wide and abundant distribution of insects has been described by explorers from the frozen arctic to the hottest equatorial regions. A volcanic island can scarcely emerge in midocean before it is inhabited by this form of life. Now, with dams and reservoirs, our own western deserts are being converted into vast breeding areas. The entire earth is filled with a terrestrial and aquatic insect fauna that lives and multiplies and dies to litter the surface and contaminate the air.

The normal population, not counting swarming or unusual increases, is at best roughly estimated for the insects living in soil. Counts in grassland in England, made to a depth of 9 to 12 inches of soil and including mites, numbered several hundred million per acre. In an oak and pine stand in North Carolina, 120 million per acre were found to a depth of 5 inches.¹

Composition of aerial insect fauna reported by Freeman²⁰ showed, during a period of study, a total of 23,295 specimens represented by 421 species. Of these, 75 per cent were below 100 feet. *Diptera* were most numerous below 100 feet, while *Hymenoptera* were most numerous above this level. *Aphidae* was the dominant family at all heights. The greatest number and varieties occurred in June and September.

Of the *Hexapoda* alone, the kinds number more than a million. About two-fifths are beetles and another two-fifths include the *Lepidoptera*, *Hymenoptera*, and *Diptera*. The remainder comprise the final fifth. Sabrosky¹ further staggers the imagination with a few colorful observations. About 40,000 cicadas have been observed to emerge from the ground under a large tree; a queen bee may lay 2,000 eggs a day; and some termites are capable of laying an egg every two seconds, at which rate, a termite could lay a total of 43,000 eggs in a single day. A number of insects add to this prodigious multiplication by polyembryony (two or more young from a single egg). Parasitic wasps, for example, may have several thousand young emerge from a dozen eggs.

Fourth and Fifth Postulates.—These postulates are more adequately fulfilled for insects than for other aero-allergens. Not only are most insects capable of purposive (active) migration, but perhaps more important to us is the passive dispersion of these arthropods. There is scarcely an insect that at some time in its life cycle does not take to the air. Just as the ocean is adrift with marine plankton, so the air above may be filled with insect fauna that might well be named "aerial plankton."

The actual dispersal of insects is so extensive that there is less need to be familiar with those of any specific location than in the case of pollinating plants. The cotton moth of the South appears in large numbers in Canada, while the monarch butterfly, which allegedly cannot survive above subtropic temperature,

is found in large quantities as far north as the Arctic Circle and out into islands of the Pacific. Arachnids, such as spiders and gall mites, travel thousands of miles on trade winds and can be found, by air sampling aboard ships at sea, in numbers which, if applied to pollen grains, could be of clinical significance. Migrations of butterflies are especially striking. Millions of these *Lepidoptera* may fly in a swarm several hundred miles in width and traverse several thousand miles¹ over a period of days.

Felt,³¹ who contributed a great deal toward a better understanding of this dispersion of insects, aptly stated: "Insects took to the airway back in the Upper Carboniferous Period, and it is not surprising that Man, a most recent traveler upon the 'wings of the wind,' should find much that is new, especially in relation to the insect inhabitants."

Dissemination of insects by air currents has been of interest to those particularly concerned with the economics of entomology. However, this may prove of significance in accounting for seasonal flare-ups of respiratory allergy. Felt,³² Glick,³³ Coad,³⁴ Wolfenbarger,³⁵ and others described the dispersion of insects and other small organisms and made counts of the population at various levels above the earth's surface. Coad,³⁴ collecting insects from the level of 50 to 14,000 feet, found the densest population up to 1,000 feet. This was reduced to one-half at 2,000 feet and further reduced to one-half at 3,000 feet. After 6,000 feet, the population was lightest. Contrary to first thought, the strongest-flying insects were located closer to the ground while the smaller, weaker ones were found at higher altitudes. Wingless insects were collected at high altitudes of more than 10,000 feet. It is evident from this that travel and dispersion were partly voluntary and to a great extent involuntary through the rising air currents.

Glick³³ and Felt³² enumerated meteorologic conditions which might affect and thus even predict the concentration of insects:

1. *Temperature.* This is most important. The optimum is 75° to 80° F. There is a rapid drop in count with temperatures over 90° or under 70° F.

2. *Humidity.* Numbers increase as absolute humidity rises. Optimum dew point is about 62° F.

3. *Barometric pressure.* This has a varying effect. Some insects become nervously active at low pressures (approaching storms); others swarm at higher barometric pressures. Crickets and other insects are often found in vast numbers on the western shores of Lake Michigan after a storm.

4. *Wind velocity.* This is also a complex subject. Insects, especially moths, migrate with the prevailing winds that are favorable. Winds of high velocities at lower levels are of minor importance. Convection currents and other upward air streams carry large numbers of insects (especially those weak of flight) to high altitudes of 15,000 feet and more, where horizontal wind velocities may be 100 to 200 miles an hour, carrying this "aerial plankton" many hundreds and even thousands of miles.

The physiographic aspect of a region affects the total insect count. Streams, lakes, oceans, and mountains³⁶ contribute much to this. The winds follow valleys and rivers. A change in temperature of the insect-laden winds may cause large numbers to appear on the edge of a lake in a mountain resort where hay fever sufferers have sought refuge. Temperature and moisture affect not only the growth of insects but also the vegetation upon which they feed. Thus, in his deforestation and extensive irrigation projects, man has indeed altered the flora and at the same time the insect fauna.

The invasions of homes by insects are frequent experiences that may call for thorough fumigation by professional exterminators. The occurrence of asthma as a result of such invasions has been reported.^{9, 21, 22} The more casual occurrence of insects in homes and places of work may presumably be even more prevalent. Felt and Chamberlain³⁷ studied this problem in tall office buildings. Every³⁸ reports the steady invasion of homes by many insects seeking warmth from approaching inclement weather. Among these invaders he includes strawberry root weevils, grass weevils, box-elder bugs, grass bugs, and leaf hoppers.

This discussion of dissemination and concentration of insects has not emphasized the insect debris that must accumulate from successive generations that have bred and died and become a part of our dust in a single season. In sheltered areas, as well as in homes, this may become an accumulative factor of several seasons.

The great obstacle in measuring this aerial fauna and the insects within homes is the lack of a method for readily identifying such insect debris by air sampling in a manner comparable to that used for pollens. Slide counts of insect hair, scales, and other debris³⁹ have been of little clinical value. Identification of air or dust samples by other physical means has been disappointing. Detection of the insect cuticle by fluorescence and phosphorescence cannot be considered distinctive since other compounds, including albumins, gelatin, and keratin, are similarly fluorescent.⁴⁰ Spectroscopic examination of dust samples containing insects has likewise given little promise. Thus far, only a few studies have been done with the absorption or transmission of infrared irradiation. When some technique is devised for even a gross quantitative estimate of insect material in the air and dust, then we may feel that we are moving forward to the better evaluation of insects as inhalant allergens.

NATURE OF INSECT ALLERGENS

General Consideration.—Little is known of the nature and number of excitants that produce immediate whealing and are capable of producing antibodies that may readily be passively transferred. There is reason to believe that two or more such excitants exist. It is important to know whether these are common to the entire Phylum *Arthropoda* or are specific for the class, for the order, for the family, or even for the species. Gaillard,¹⁰ working with aphids, felt that the protein in the exoskeleton varied with insect species and, since he presumed that the protein was the antigenic element, he concluded that insect allergens were species specific. This is, indeed, a frightening presumption

and it is hoped that a common denominator can be found to permit grouping of such allergens by families or, even more broadly, by order within each class in the Phylum *Arthropoda*.

Chemistry.—The integument of the *Arthropods*, like the epidermal tissue of birds and mammals, is generally presumed to contain the allergenic substances. In preparing insect allergen, however, the entire organism usually has been used—thus throwing some doubt on the propriety of applying our knowledge of the chemical nature of the insect cuticle to the allergenic extract used in most clinical studies, even though the wings and exoskeleton of most insects make up the great proportion of the dry weight of these organisms. Nevertheless, some light may be shed by reviewing what is known of the chemistry of this exoskeleton.

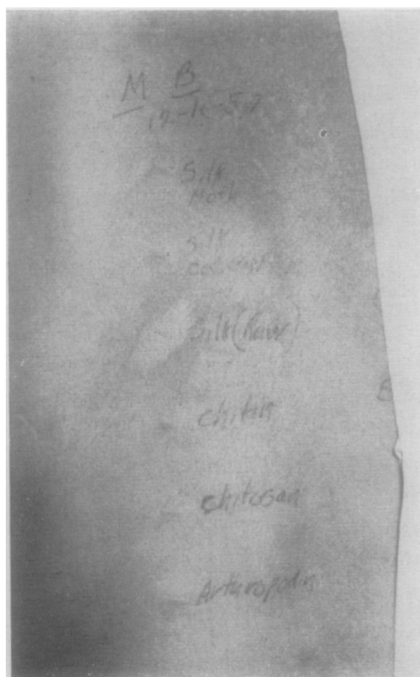


Fig. 3.—Strong reaction to silk. Moderate reaction to silk moth and silk cocoon. Negative reaction to chitin and arthropodin.

The insect cuticle possesses protein, carbohydrates, and sulfur-containing materials.^{41, 42} The proteins, which are of most interest, are generally large and chemically possess similar properties for many of the insects studied. These proteins change in the course of development from the larval to the adult stages. For example, their solubility is greatly reduced during pupation. Similar chemical properties, however, cannot be literally translated as allergenic similarity. The substances chitin, arthropodin, and sclerotin have been identified in varying proportions in the cuticle of most insects.

Chitin.—This substance has been implicated frequently in insect allergens;¹⁰ yet, this is only one component comprising from one-fourth to one-half of the dry weight of the entire cuticle. Although some investigators⁴³⁻⁴⁵ have studied the protein in chitin and it has been considered to exist in minute amount as a polysaccharide-protein complex, there is much evidence that would cast doubt on the probability that chitin is implicated in the allergenicity of insect extracts used in clinical testing (Figs. 3 and 6). It is quite probable that allergists are erroneously using the term *chitin* in place of the term *cuticle*. Chitin is quite insoluble, resembling cellulose in this respect (and in others). Chitin, for example, differs from silk in that the latter is secreted by glands derived from the epidermal layer, while chitin itself, although crystallized in a similar way, is secreted by glands of hypodermal origin, resembling synovial fluid of the vertebrates. This may explain why both chitin and synovial fluid are capable of calcification. It must be presumed, however, that although chitin is extremely insoluble, some changes must occur in nature. The minute copepod crustacea produce several billion tons of chitin annually,⁴⁶ while other marine crustacea and the terrestrial arthropods produce several times this astronomic amount. It is evident that if this insoluble substance and other components of the cuticle were not decomposed, soon the earth's available elements, especially carbon and nitrogen, would be seriously depleted. Evidence of chitinoclastic activity by a variety of bacteria and fungi through enzymatic action has been demonstrated by a number of authors.^{47, 48} Whether or not an intermediate product in this breakdown is allergenic remains a moot question.

Chitosan.—This is one of many derivatives of chitin and is produced by fusion with alkali at high temperatures. It does not occur naturally. It has low nitrogen contents (Kjeldahl). Results of clinical testing with chitin and chitosan will be discussed subsequently.

Arthropodin.—The proteins of the insect cuticles which are presumably the allergenic substances have been inclusively called by the ill-defined term *arthropodin*. Many investigators recognize several protein fractions. Fraenkel and Rudall⁴⁵ first proposed to this water-soluble fraction the name *arthropodin* "on account of its universal occurrence among arthropods' limbs." Hackmann⁴⁹ and his associates, in recent work, have shown that arthropodin is heterogeneous and that arthropodins from different insects give different electrophoretic patterns. They believe that there may be some protein fraction or fractions common to all insects. Their proteins extracted by the borate buffer and those extracted by water are not (as formerly thought) identical. They found that water-soluble protein may account for as little as 15 per cent or as much as 50 per cent of the protein in the insect cuticle. The arthropodin made available for our studies was a protein extract from larval cuticle of *Diaphania dorsalis* Don., prepared by a borate buffer of pH 9.

This group of proteins has certain characteristics:

1. It is quite soluble in water.
2. It does not coagulate in hot water.

3. It is precipitated by ethyl chloride above 45 per cent and by one-half saturated ammonium sulfate and saturated sodium chloride as well as cold trichloroacetic acid.

4. After precipitation by trichloroacetic acid and alcohol, it is readily redissolved.

5. Boiling produces molecular configuration of denaturation, but the protein remains soluble in water.⁵⁰

6. Partial analysis of arthropodin shows high tyrosine and low glycine but no amino acids containing sulfur. In this way it resembles sericin.⁴⁰

Sclerotin.—The commonly occurring water-insoluble protein was termed *sclerotin*.⁴⁴ This substance can be removed by 5 per cent potassium hydroxide solution. The soft cuticle contains a high percentage of water-soluble fraction; in the hard cuticle, however, the larger percentage is represented by this water-insoluble protein. Sclerotin is still not well understood, although it is thought by some to be a derivative of arthropodin in the development of the insect. It contains less nitrogen but, on the other hand, it does contain sulfur and carbohydrate. To the allergist, this is an important point. If the proteins of the larval or pupal stages are allergenically identical to that of the adult stage (but a more water-soluble protein is available), then it would be advantageous to prepare the allergen from the preadult stage of the insect to be studied. Benson,⁵⁶ however, in studying hypersensitivity to the mosquito, found that although the newly hatched species reacted in a manner similar to the reaction obtained with the older insects, the pupae failed to incite skin reactions in a sensitive patient. Further studies on other insects in various stages of development are in progress.

What part these proteins play in the total allergen is not entirely clear. In the case of arthropodin, several insects (*Crustacea* and *Arachnida*) contain this water-soluble fraction with the same general characteristics⁴⁴ and have similar chemical and electrophoretic patterns.⁴⁹ Such a finding might stimulate a further search for the common allergens.

Silk.—This cannot be included, in the strict sense, in the insect cuticle, although it is produced by cells of ectodermal origin homologous with the simple epidermal cells that produce various surface structures, including hair and cuticle. Silk is produced by many insects, including spiders, *Lepidoptera* (larval stage), and most other orders within the class *Hexapoda*. These include *Trichoptera*, *Siphonoptera*, *Hymenoptera*, some *Coleoptera*, *Diptera*, *Homoptera*, *Corrodentia*, *Neuroptera*, and *Embüdina*.⁴⁰ This number may still be incomplete, but the listing of so many common insects capable of producing a silk may well account for the frequency with which some patients react to almost all of the insect allergens used in testing (Fig. 1). Silk may be, after all, but one allergenic link between insects.

The raw silk produced by the larva of the silk worm moth, *Bombyx mori*, consists of about 75 per cent fibroin and 25 per cent sericin (silk gelatin). The sericin which acts as a cement for the two strands of fibroin to produce the

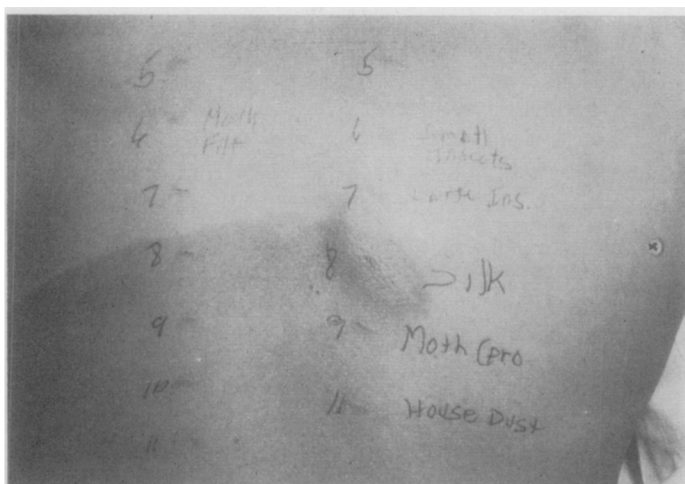


Fig. 4.

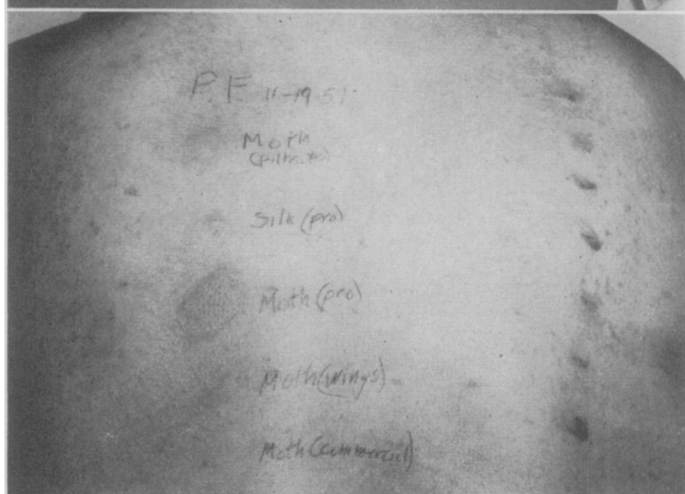


Fig. 5.

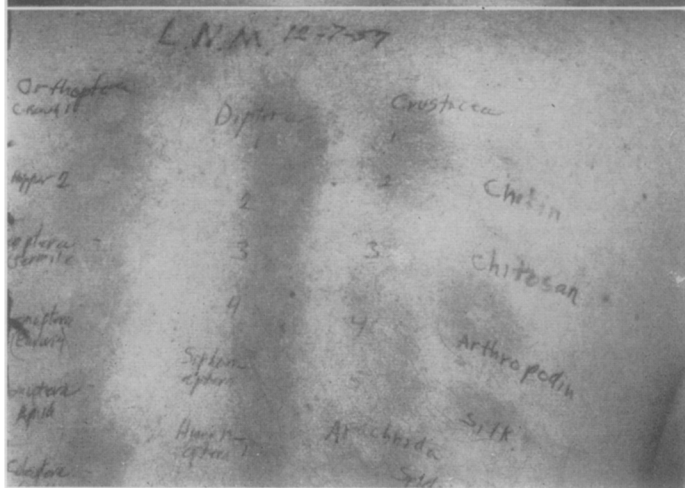


Fig. 6.

Fig. 4.—Large reaction to silk. Negative reactions to all insects, house dust, and epidermals.

Fig. 5.—Strong reaction to moth "protein." Moderate reaction to moth filtrate. Negative reaction to silk. Moderate reaction to moth-wing solution prepared eleven years previously (October, 1946). Negative reaction to moth extract from commercial laboratory (solution made in October, 1957).

Fig. 6.—Strong reactions to arthropodin and to insects. Only slight reaction to silk and negative reaction to chitin.

silk filament is water soluble and removed in the course of processing the fiber. With its lack of commercial value, it has received little study as to its chemical nature. Recently, however, it has been found to contain two separate albumins with different physical and chemical properties.⁵¹ Such findings certainly do not help to simplify the search for a common allergen in the silk of the various insects. Recent reports by Coleman⁵² and by Friedman and associates⁵³ of severe allergic reaction to a vaccine filtered through silk add emphasis to its allergenic properties. Presumably, the water-soluble sericin was not entirely removed or some other allergenic substance was responsible. (Their inability to exhaust the sensitized site with silkworm extract, however, permits too many possibilities to offer any rational interpretation.)

It is well to point out again the hazard involved in applying chemical studies to allergenicity as demonstrated by skin testing on sensitive patients or passively sensitized skin sites. The skin test demonstrates differences in the various water-soluble and water-insoluble fractions from the whole insect and from insect cuticle. Figs. 4, 5, and 6 show reactions to silk only, to the whole insect only, and to arthropodin and not to silk, among many other variables in the course of patient testing. Chitin and chitosan failed to react on all occasions.

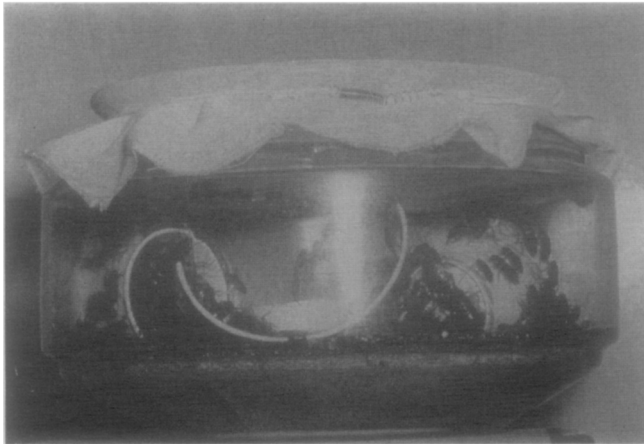


Fig. 7.—Breeding jar for propagation of cockroaches in large numbers.

SOURCES OF INSECTS

The gathering of insects required many hands to provide representatives of the various classes, orders, families, and even species within the Phylum *Arthropoda*, and in sufficient numbers to permit the preparation of an adequate supply of extract. Some were grown in the laboratory. These include cockroaches (Fig. 7), mosquitoes, flies, ticks, parasitic wasps, and fleas. If the need should warrant, perhaps many others could be thus provided. Other insects were gathered in large numbers on a single field trip. This was particularly true of the swarming varieties. Those insects that live solitary lives required

more patience and many additional hands. The neighborhood children proved enthusiastic assistants, who, in the course of an afternoon field trip, gathered large numbers of insects which were later segregated.

Insects within the Class *Crustacea* were acquired in various ways and included *Daphnia*, minute brine shrimp (plankton), and sow bug or pill bug (*Armadillidium vulgare*). The *Daphnia* were readily gathered with a dip net from a large stagnant casting pool. The catch was especially abundant in late spring and early autumn. The brine shrimp were raised in myriads from eggs purchased in a tropical fish store. However, this plankton can be obtained freshly frozen from this same source. Sow bugs are abundantly present in damp areas under rocks and logs and in places where wood touches moist dirt. On a single Sunday afternoon, even in early spring, many thousands were quickly gathered, leaving the impression that their bodies and skeletons must certainly contaminate the uncultivated earth and the unexcavated cool, damp areas beneath homes.

A practical and inexpensive apparatus for gathering large quantities of insects is the light trap. In a short period of several nights, a pint of insects was caught (Figs. 8 and 9). The process of separating them according to orders was tedious but less time-consuming or costly than attempting to collect so many in the field. The greater share of this material obtained in the light trap was processed without segregation, labeled "mixed insects," and provided excellent material for screening patients for later detailed testing and for specific treatment. A highly allergenic and concentrated "protein precipitate" was made from one such "mixture" of insects.

In the past, handling insects before preparing the final extract was simply a matter of storing under desiccation the various specimens gathered. Osgood¹⁶ describes collecting his caddis flies in a live state during flight, as well as sweeping up large quantities that had dried in the summer air. Evidently both provided excellent sources of allergenic extract. This insect debris, after several months in a dried state, was still capable of inducing asthmatic symptoms in the sensitive patient who chanced to inhale it. Gathering and storing in the simple dried state such insects as caddis fly, May fly, housefly, mosquito, and moth may be satisfactory. However, any slight amount of moisture will quickly result in mildew and make worthless the entire contents. In storing the whole insect, bacterial action may continue, resulting in some putrefactive changes so that most insects stored as they are gathered, even under desiccation, soon develop the characteristic unpleasant odor. This is particularly true of the large-bodied insects, such as the beetle, grasshopper, bee, hornet, and yellow jacket.

To avoid this spoilage, the method was followed of processing from the live stage or quick freezing and storing insects under subfreezing temperature.⁵⁴ The latter method of storage was necessary for those specimens that could be gathered only a few at a time (spiders, solitary wasps, etc.). Insects processed from the live or quick-frozen stages yielded a final product that was essentially odorless.

Fig. 8.

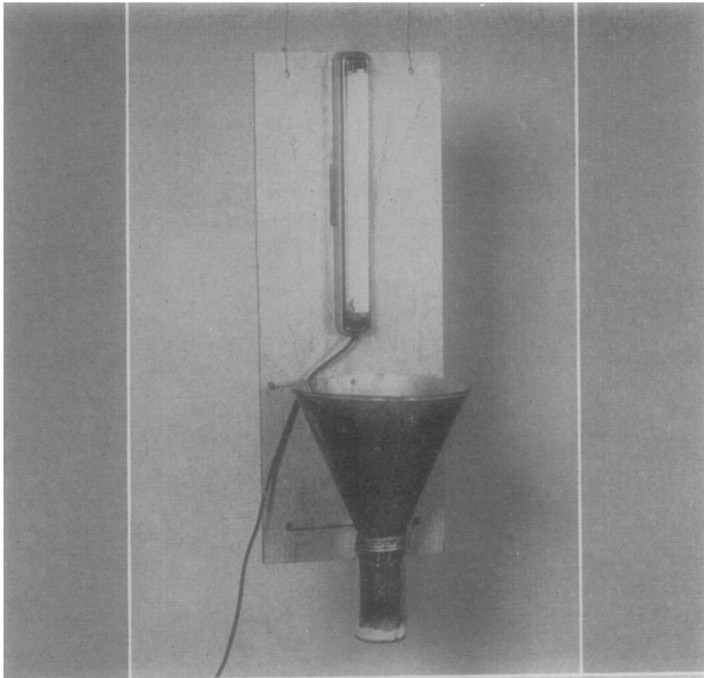


Fig. 9.

Fig. 8.—Light trap, fluorescent tube, and funnel through which insects drop into screw-top jar with inner wall screen. At bottom of jar is gauze soaked with carbon tetrachloride.

Fig. 9.—Three-day "catch" in light trap (almost one pint by volume). Most of the common important hexapods are represented.

PREPARATION OF INSECT ALLERGENS

The procedure in preparation of these allergens must necessarily vary with each type of insect used. Among the many reports in the literature of testing and treating with insect allergens, usually perfunctory comments are offered as to their preparation. In most cases it must be presumed that a simple concentrated filtrate was used. Gaillard¹⁰ defatted aphids with toluene and extracted with Coca's solution. This extract was further dialyzed, filtered, and standardized according to its total nitrogen content. He reports 0.16 mg. total nitrogen per milliliter (T.N./ml.), which is rather low in comparison with analyses of our extracts. The dark brown color he describes, if gradual in development, may have been the result of the action of phenol in Coca's solution and must be reckoned with in the stability of this aqueous allergen. Osgood,¹⁷ working with the Caddis fly, also defatted and finally standardized the aqueous filtrate on the basis of protein nitrogen. Frankland,⁵ working with locusts, defatted with acetone and extracted with buffered saline. He found the feces of locusts a potent allergen. Benson and Semenov,⁵⁵ working with the honey-bee, prepared separately from the body and from the stinging mechanism a "protein" precipitated out by saturating the aqueous extract with magnesium sulfate. Dialysis for thirty-six hours gave two fractions—soluble and insoluble, respectively—which they presumed to be the albuminous and the globulin fractions.

It is evident that, despite the diverse techniques, similar fundamental principles are employed: first defatting and then extracting with isotonic buffered aqueous solution. The final extract obtained is usually concentrated. Such extracts, in addition to being water soluble, are insoluble in alcohol, nondialyzable, and thermostabile,⁵⁶ and contain no significant nonspecific irritants. The protein-nitrogen content is appreciable and is often used as a guide in standardization. No studies have been done on the stability of these extracts. Since the entire body of the insect is used, it is possible that certain enzymes may be carried through during the extracting process, thus later slowly altering the active protein to a split nonprotein product which has less allergenicity. Therefore, perhaps a dried or lyophilized state would offer material that would be or remain stable and could be stored for an indefinite period of time.

The method of preparing insect allergen material used in our study has been repeatedly modified over a period of several years, but it has recently been standardized. All insects processed from a live or quick-frozen state are defatted with ethyl ether—some with low fat contents (*Orthoptera*) by simple overnight soaking and others with very high fat contents (*Hymenoptera*) by Soxhlet's apparatus. The insect bodies must best be broken up to obtain thorough defatting. Without adequate removal of the fatty substances, filtering of the final aqueous extract proved tedious and discouraging. After defatting, this is further ground by hand with mortar and pestle and placed in a flask to be lyophilized for twenty-four hours. The material is then finely ground in a

Wiley mill, using a 60- to 80-mesh screen, following which the fine powder is placed in the lyophilizer for another twenty-four hours. The final product is stored in a desiccator indefinitely without fear of mildewing or deteriorating.

Fig. 10.

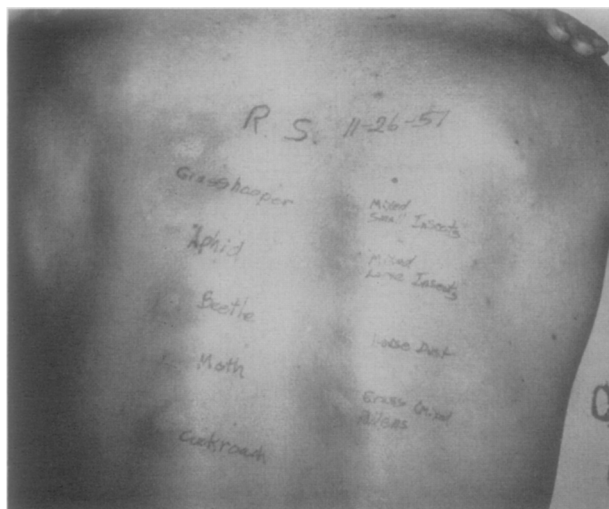


Fig. 11.

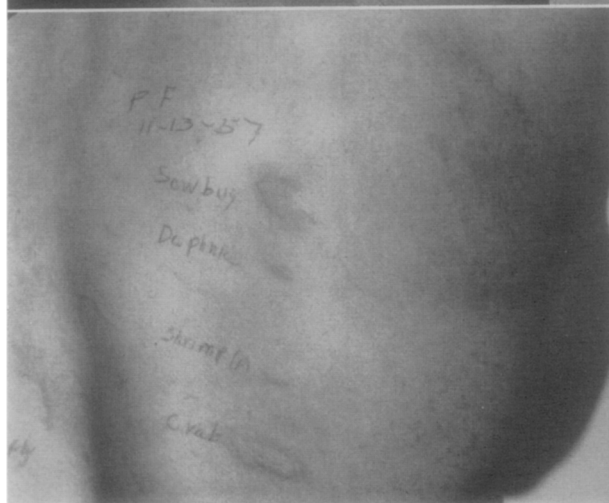
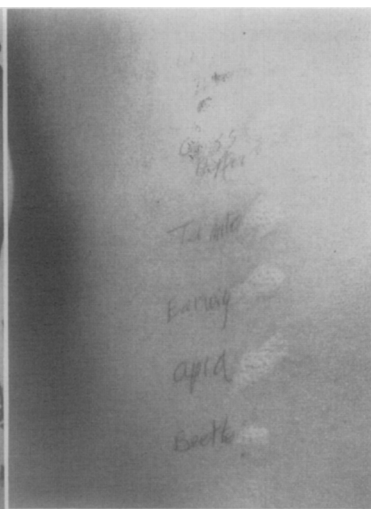


Fig. 12.

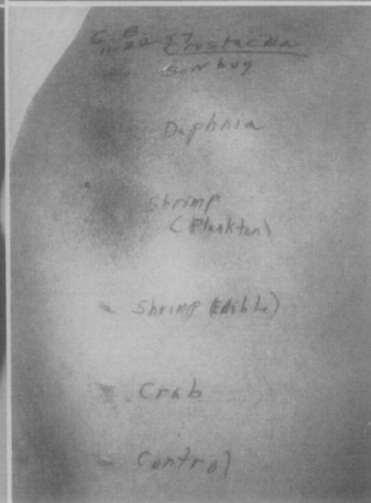


Fig. 13.

Fig. 10.—Selective reactions to insects and to pollens. Negative reactions to aphid, moth, and house dust.

Fig. 11.—Selective reaction to several *Crustacea*. Strongly positive to sow bug and crab. Slight reactions to shrimp and *Daphnia*. (The reverse is seen in Fig. 12.)

Fig. 12.—Moderate positive reaction to minute fresh water and salt water *Crustacea*. Negative reactions to other *Crustacea* (the reverse of Fig. 11).

Fig. 13.—Positive scratch reactions to termite and earwig, among others.

A few insects were gathered in sufficiently large numbers to permit the preparation of a "protein powder." This was made by adding to the crude aqueous filtrate four parts "ice cold" acetone. The precipitate was dried and pulverized and made ready for redissolving in small amounts as needed. This

procedure was followed in preparing the "protein precipitate" of moth, mosquito, bee, and a "mixture" of small insects recovered from the light trap. As was expected, powdered precipitate in the case of each insect had a higher protein-nitrogen content and a stronger whealing effect than simple aqueous extract (Fig. 5). It is an ideal procedure of course, for preparing good insect allergen but is practical only when an adequately large quantity of original material can be gathered.

For later preparation of an aqueous extract, a small weighed amount of lyophilized fat-free material is dissolved in ten parts buffered 50 per cent glycerin by weight to volume and extracted for forty-eight hours at room temperature, shaking several times a day. (Some insects require greater dilution.) This is then centrifuged slowly and the supernatant solution is passed through a sterilizing Seitz filter. A portion of the final filtrate is used for scratch testing. The remainder is stored in sterile bottles for further dilution to be used in intradermal testing and treatment. This glycerinated buffered extract has, for some insects, retained its allergenicity now for many years. For example, with such extracts of moth wings in buffered glycerin, a strong whealing reaction was obtained by the scratch method after eleven years (Fig. 5).

The method thus outlined in some detail for preparation of concentrated filtrate is not at all laborious. It is superseded in quality only by precipitating out the "protein" with cold acetone. However, the latter procedure for the present is practical only with a few insects. If, in the future, it is found that some allergen or allergens common to all or many insects exist, then a precipitation method of preparing a "purified protein" can be used on insects that can be gathered in large quantities to yield such basic materials. As evidenced in these studies, arthropodin, silk, and chitin still do not account for all the allergenic substance in the insect; nor can we depend on tests with one order to be interpreted as tests of sensitivity for another (Figs. 10, 11, 12, and 13).

BIOCHEMICAL ANALYSES OF INSECT ALLERGENS

Total nitrogen and protein-nitrogen studies were not done routinely, but Table II shows determinations on several unselected extracts prepared as filtrates and as "protein precipitates." The samples used for analysis were in 50 per cent buffered glycerin which had been passed through a sterilizing Seitz filter and was ready for skin testing. Each of these samples was submitted to three analyses. Phosphotungstic acid was used to prepare them for nonprotein-nitrogen determination. After Kjeldahl digestion, the flask contents were made alkaline and the ammonia steam was distilled into boric acid which was then titrated further against 1:100 normal hydrochloric acid. There was no more than 6 per cent variation in these three analyses, and the average was recorded in Table II. The "protein precipitate" in a dilution of 1:25 by weight volume, as would be expected, showed higher total and protein nitrogen contents. The large-bodied insects, such as the cockroach and grasshopper, as simple filtrates in a 1:10 dilution approximated the more concentrated extracts in the protein-nitrogen contents. The nonprotein-nitrogen contents in all samples accounted for more than one-half the total nitrogen determination of this water-soluble

material. Quite probably that does not represent alterations of the protein. We are dealing here with a crude aqueous extract of the whole insect, and a better understanding of this nonprotein nitrogenous material must await our further studies.

TABLE II. NITROGEN CONTENTS (PROTEIN AND NONPROTEIN) OF CERTAIN INSECT ALLERGENS AND SILK

ALLERGEN	DATE SOLUTION MADE	DATE OF ANALYSIS	TOTAL NITROGEN (MG./ML.)	NON- PROTEIN NITROGEN (MG./ML.)	PROTEIN NITROGEN (MG./ML.)
Silk (raw) "protein" 1/25	11-23-57	12-17-57	1.500	0.215	1.285
Small insects (mixed) "protein" 1/25	12-16-57	12-17-57	4.497	2.820	1.677
Small insects (mixed) filtrate 1/10	4-10-57	12-17-57	3.070	2.285	0.785
Moth "protein" 1/25 ("old")	10- 8-56	12-19-57	1.643	1.000	0.643
Moth "protein" 1/25 ("fresh")	12-16-57	12-19-57	2.285	1.248	1.037
Moth filtrate 1/10	12-16-57	12-19-57	2.070	1.535	0.535
Moth filtrate 1/10 (commercial lab.)	10-25-57	12-19-57	0.7150	0.5700	0.1450
Cockroach filtrate 1/10	12-18-57	12-27-57	3.177	2.213	0.964
Grasshopper filtrate 1/10	12-18-57	12-27-57	3.177	1.713	1.464

Analyses of the four samples of moth extracts revealed several interesting points:

1. The "protein precipitate" contained more protein nitrogen and less nonprotein nitrogen than the simple filtrates.

2. "Protein" prepared on Oct. 8, 1956, and that prepared one year later on Dec. 19, 1957, were from the same dried powdered material but were prepared separately in solution form; thus, a positive statement of reduction in protein nitrogen cannot be made.

3. It is interesting to note that of the two "protein" samples there was no striking increase in nonprotein nitrogen after fourteen months, which may be some index of stability of this material in buffered glyc-erinated solution.⁵⁸

4. The moth filtrate from a commercial laboratory was much lower in protein nitrogen and relatively high in nonprotein nitrogen. This is reflected in the lack of whealing on known sensitive patients (Fig. 5).

It must be reiterated that these analyses can offer no fundamental conclusions but are early and interesting observations made in the course of our studies.

CLINICAL STUDIES

Abundant clinical and immunologic evidence has shown that a number of insects act as inhalants to produce symptoms of hay fever and asthma (Table I). An attempt at clinical correlation of such symptoms with the rest of the

insect fauna, with its emanation and with insect debris, becomes a more difficult task. Many conclusions must be drawn by inference, while the final acceptance will have to await the results of vast clinical experiences over a period of years.

As patients are studied more thoroughly with the thought that most insects may be inhalant factors, much provocative evidence is revealed. This clinical problem has been passed over lightly—not only because of failure to use the insect allergens more routinely in testing of patients but, more important, because the few such allergens available commercially have often proved virtually inert. This was confirmed recently after testing known sensitive patients with some of these allergens of questionable worth (Fig. 5).

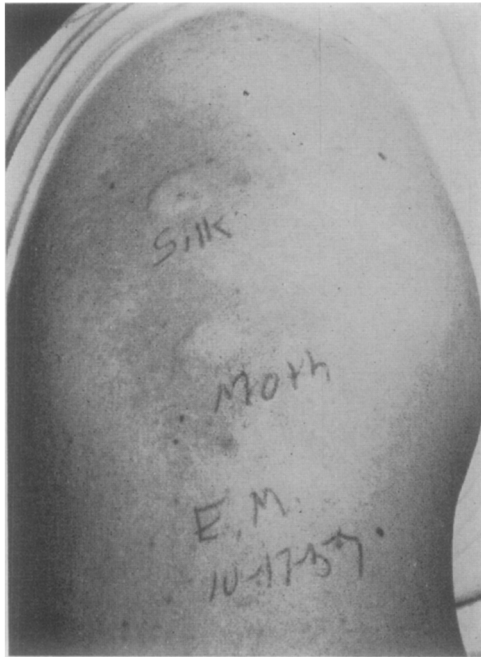


Fig. 14.—Typical reactions to both silk and moth extracts.

At the onset of these studies, testing was performed only on those patients with a history of seasonal symptoms but with negative reactions to pollens. In none of these twelve or more patients were there significantly positive reactions to insect allergens. Next, it was found that patients sensitive to silk often reacted to such insects as moths (Fig. 14), while those sensitive to crab and lobster, reacted to *Daphnia* and to the minute ocean plankton (shrimp). In these patients, when more insect allergens were tried, frequently many positive reactions resulted (Fig. 15).

The major portion of our clinical studies, however, is based on routine testing with insect allergens on consecutive unselected patients who were referred because of a history of allergy or presumptive evidence of an allergic disorder. The number and variety of insect allergens applied increased in the course of

results. The first 205 cases, therefore, are not included in the tabulation to follow. The remaining 252 consecutive patients studied provided more concrete conclusions, and eighty-three of those patients are now receiving injections of insect allergens. At the conclusion of these studies, the records of the last 252 patients were tabulated for the following:

1. Age and sex incidence.
2. Geographic distribution, occupation, and race.
3. Relationship to clinical diagnoses.
4. Relationship of skin reaction to insects and to other inhalant allergens.
5. Interrelationship of insect allergen reactions.

In general, it was found that approximately one-half of the patients tested routinely by the scratch method reacted to several of the insect allergens (Table III). When intradermal testing was done to confirm equivocal scratch tests, the percentage of positive reactions rose above 65 per cent. In that group on whom only intradermal testing was done, the percentage of positive reactions was understandably low. These patients were tested directly by the intradermal method because they were presumed not to be reactive to any allergens. Most of them were in the older age groups. In some instances we were surprised by very large local and several systemic reactions.

TABLE III. INCIDENCE OF SKIN REACTIONS TO INSECT ALLERGENS
(ANALYSIS OF 252 CONSECUTIVE UNSELECTED PATIENTS)

METHOD OF TESTING	TOTAL TESTED	NEGATIVE TO $\pm$	+ ++	+++ ++++	TOTAL POSITIVE	PER CENT POSITIVE REACTION
Scratch only	41	20	3	18	21 + - ++++	50
Scratch and intradermal	86	56	11	19	30 + - ++++	35
		19	10	57	57 +++ - ++++	66
Intradermal only	125	51	30	39	39 +++ - ++++	31

Sex, in relation to insect allergen, might have reflected more the difference in occupation and exposure of patients to insect debris. However, there was a remarkably even division between the sexes as to those who did and those who did not show reactions (Table IV). This again may point to the ubiquitous nature of the insect allergen.

Age variation is shown roughly in Table V. Although small children were often tested by the scratch method only, and those in older age groups by intradermal methods directly, some gross observations can be made. It is evident

TABLE IV. INCIDENCE OF POSITIVE REACTIONS TO INSECT ALLERGENS IN RELATION TO SEX

SEX	TOTAL	NEGATIVE	POSITIVE
Males	146	74	72
Females	106	53	53

that, although about 50 per cent of the entire group showed positive reactions, those in the early age groups and those over 45 years of age fell much below this, while those between the ages of 16 and 35 showed a frequency of reactions to insects well above the 50 per cent average.

TABLE V. INCIDENCE OF POSITIVE REACTIONS TO INSECT ALLERGENS IN RELATION TO AGE

AGE (YEARS)	TOTAL TESTED	SCRATCH ONLY		SCRATCH AND INTRADERMAL		INTRADERMAL ONLY		TOTAL	
		NEG.	POS.	NEG.	POS.	NEG.	POS.	NEG.	POS.
5 and Under	14	8	—	—	4	—	2	8	6
6-15	47	7	5	10	12	4	9	21	26
16-25	27	—	3	3	11	2	8	5	22
26-35	44	1	6	5	12	9	11	15	29
36-45	53	5	3	2	11	22	10	29	24
Over 46	67	3	1	7	11	28	17	38	29
Total	252	24	18	27	61	65	57	116	136

GEOGRAPHIC DISTRIBUTION OF PATIENTS SENSITIVE TO INSECT ALLERGENS

The patients studied were distributed over a vast area of the Pacific Northwest, including the states of Oregon, Washington, and Idaho. There were also a number of patients from northern California, Nevada, and western Montana. All varieties of climate, altitudes (from sea level to 8,000 feet), occupations, and population densities were represented. The population in the Pacific Northwest has increased in the past ten years by more than 40 per cent, representing migration from all corners of the United States, Central America, Europe, Africa, and the Orient. Patients from London, England, to Siam, and from Ghana to Melbourne, Australia, are represented and showed reactions to insect allergens.

In these studies, all patients from the above-enumerated areas of the Pacific Northwest were proportionately represented without any striking predominance of any localities or occupations. In spite of these diverse meteorologic conditions and racial groups, no one group revealed any higher percentage of patient reactions to insect allergens than another. This is certainly in contrast with pollen sensitivity, which conforms with the flora of the region in which the patient is living or has lived for a number of years. Perhaps a lack of conformity of insect-sensitive patients to race and region is further evidence of the universal distribution of orders of the *Hexapoda*, as well as other classes of arthropods.

It has, been reported of course, that in areas in which insects appear abruptly and in large numbers, these insects prove to be responsible for acute symptoms. This is best exemplified in the Great Lakes areas (caddis fly and May fly). Such proved seasonal problems should stimulate more intense investigation in other areas that may well produce such allergenic conditions in less obvious proportions. The cicadas (*Homoptera*) in the Midwest, the lady

beetle (*Coleoptera*) in the Southwest, the inevitable moth and butterfly (*Lepidoptera*), and the cricket, grasshopper, and locust (*Orthoptera*) in other areas which are periodically plagued will merit closer scrutiny.

Another area for investigation might well include those regions where the minute brine shrimp is abundant. This would designate the San Francisco Bay

Fig. 16.

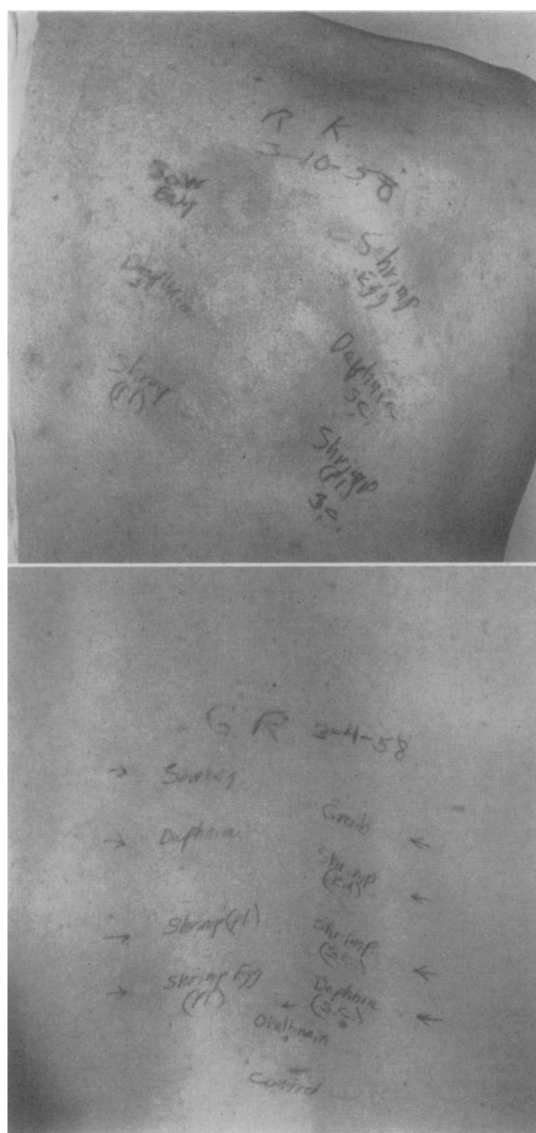


Fig. 17.

Fig. 16.—Strong reactions to all *Crustacea* extracts tested. Negative reaction to shrimp (plankton) eggs.

Fig. 17.—Strong reaction to shrimp (plankton) eggs. Negative reaction to all *Crustacea* extracts and ovalbumin.

area, the Gulf of Mexico, the Salt Lake basin, and other salt water areas where myriads of these minute organisms fill the shallower waters. In addition, their eggs, almost dustlike in size and color, which are gathered and packaged for hatching to be used as food for tropical fish, may also be inhalant factors in these areas.

Crustaceans are common ingestant allergens and violent reactions are not infrequently observed. In our studies, however, the *Crustacea* and its subclasses and orders as insect allergens were also explored. This included *Daphnia* of fresh water, minute salt water shrimp, and terrestrial crustaceans (sow bug). In general, positive reactions to this class occurred less frequently than to other arthropods, but they did occur frequently and violently in patients who were otherwise sensitive to crustacean food. In a number of circumstances positive reactions could be repeatedly produced for one member and not for another (Figs. 11 and 12). The *Daphnia* gathered from a casting pool in the early spring gave strong positive reactions not only to those sensitive to other *Crustacea*, but during the height of the grass pollen season *Daphnia* almost universally reacted on patients sensitive to grass pollens. The other *Crustacea* extracts did not react on these patients. This could not be accounted for on the basis of pollens in the water where the organisms were gathered, because the *Daphnia* was obtained before the grass or even the common trees were in active pollination. Recently, in autumn and winter months, this same *Daphnia* material reacted infrequently even in patients reacting strongly to pollens.

TABLE VI. COMPARISON OF REACTIONS TO INSECT ALLERGENS WITH REACTIONS TO COMMON INHALANTS

COMMON INHALANT		SCRATCH TESTING		INTRADERMAL TESTING			TOTAL TESTED
		NEG. TO INSECTS	POS. TO INSECTS	NEG. TO INSECTS	POS. TO INSECTS	NEG. TO BOTH	
House dust	Pos.	6	7	57	95	58	250
	Neg.	15	12	43	15		
Epidermals	Pos.	12	14	56	95	53	251
	Neg.	9	9	44	12		
Pollens	Pos.	13	13	35	68	71	253
	Neg.	8	9	63	44		

These observations are wholly without satisfactory explanation. On a recent December day that was free of pollen, *Daphnia* was again gathered and will be lyophilized and used in comparison, both clinically and biochemically, to determine whether there was a contaminant or a gradual alteration in the allergen itself by autolysis or other enzymatic action.

Reaction to salt water plankton (shrimp) almost invariably coincided with skin reactions to edible crab, shrimp, and lobster. The possibility that these minute but strongly allergenic organisms may affect ocean swimmers, such as *Daphnia* in the case of those swimming in ponds and pools,³ has been alluded to by several observers⁵⁹ and may occur more frequently than we have previously supposed.

Brine shrimp eggs which were grown, defatted, and tested as a concentrated aqueous extract reacted less frequently than the hatched insects themselves or than pollens and other inhalants. This potential allergen may react more frequently, however, in populations living in areas where the tiny eggs may be part of the atmospheric dust. Such occurrences would be isolated problems and might be readily overlooked. It might be well to do more routine testing with an extract of shrimp eggs in those areas where this material may be present.

The relationship between skin reactions to insect allergens and to the more common inhalants led to several striking general observations. Patients who failed to react even by intradermal testing to any of the inhalants or ingestants rarely reacted to the insect allergens. For the most part, large immediate reactions occurred as often with insect allergens as with other allergens (Table VI).

In Table VII correlation of skin reactions to insect allergens with allergic disorders is roughly presented. The largest number of cases is represented by perennial allergy symptoms affecting the respiratory tract. Many of these patients also had pollinosis and dermatologic manifestations of allergy of secondary importance. The cases of pollinosis without other complicating allergic disorders were relatively few because studies were begun on these 252 patients in July, after the peak of our pollen season had passed. The group with uncomplicated dermatologic disorders were, in most cases, of the "atopic" form and also in most cases reacted to environmental allergens by scratch or intradermal testing. At this time, studies with the unprocessed insects or with their oily extracts by patch test had not been evaluated. This phase of study of insects as contactants will deserve, however, thorough study.

TABLE VII. INCIDENCE OF SKIN REACTIONS TO INSECT ALLERGENS IN
RELATION TO ALLERGIC DISORDERS

ALLERGIC DISORDER	TOTAL	POSITIVE	NEGATIVE
Asthma, nonseasonal allergic rhinitis (with or without other complicating allergic disorders)	217	100	117
Pollinosis (without history of other allergic disorders)	15	8	7
Urticaria (without history of other allergic disorders)	3	1	2
Other dermatologic disorders* (without history of other allergic disorders)	16	6	10
Emphysema obstructive (without history of allergic disorders)	3	0	3

*Primarily eczema and eczematoid dermatitis in late youth and adulthood.

Skin reaction to silk, in the course of routine skin testing, is not uncommon. It occurs in a population that has been without the imported silk fabric for more than a decade. Reactions occur in children and young adults who have lived their days in a postwar environment of synthetics. Weiner⁵⁷ reports a case of bronchial asthma due to silk in a 6½-year-old girl. These were observations that aroused interest in insects as possible allergens to explain such frequent occurrences of silk sensitivity. Allergists have periodically commented on the

relationship of skin reactions to moth, silkworm, and silk. This is, indeed a frequent observation (Fig. 14). Table VIII shows a relationship that is not too reliable as a presumptive index of insect sensitivity. Strong reactions to

TABLE VIII. RELATED FREQUENCY OF REACTIONS TO INSECT ALLERGENS AND TO SILK

REACTION	NUMBER	PER CENT
Positive to insects and positive to silk	21	10
Positive to insects and negative to silk	86	37
Negative to insects and positive to silk	5	2
Negative to insects and negative to silk	118	50
Total	230	

insects have occurred with negative reactions to silk (Figs. 5 and 6). On the other hand, strong silk reactions have also been elicited with an absence of reaction to moth and any of the other insect allergens (Figs. 3 and 4). This can best be explained by the presence of several allergenically distinct substances and adds to the frustration of anyone seeking a common denominator.

TREATMENT WITH INSECT ALLERGENS

Eighty-three patients for whom insect allergens are included in the specific treatment were selected on the basis of (1) clinical history, (2) proportionately larger reactions to insect allergens than to any of the other inhalants, and (3) history of lack of adequate relief from treatment with conventional environmental allergens.

None of these patients is being treated exclusively with insect allergens. Results cannot be even grossly evaluated at this early date. An encouraging note is brought out in unsolicited reports by five such patients currently under treatment that for the first time their respiratory symptoms are well controlled. A good deal more observation and selection of patients will be required, however, to justify our inclusion of this insect allergen in our routine treatment of allergic disorders.

SUMMARY

1. Insects as inhalant allergens fulfill the postulates set down for pollinating plants causing hay fever and asthma.
2. The nature of the insect allergens is not well understood; nor is there sufficient evidence to place the specificity at the level of species or higher in the table of classification.
3. The known protein chemistry of the insect cuticle is reviewed as it might be related to insect allergens and includes specific comments on chitin, arthropodin, and silk.
4. Insect materials for our studies were obtained by field and light trap "catches" and by breeding in the laboratory.
5. Handling of insect material is described, and the need for prompt processing or quick freezing is discussed.

6. The method of preparation of insect allergens at first varied greatly, but a standardized technique was established and is described in detail.

7. Clinical studies are tabulated on 252 consecutive unselected patients in relation to age, sex, race, geographic distribution, occupation, clinical diagnosis, and sensitivity to other inhalant allergens.

8. Treatment with insect allergens received only few brief comments, since it is too early to draw any conclusions on results.

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