THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 46, No. 5

Printed in U.S.A.

STUDY OF THE TOXIN FROM THE POISON HAIRS OF *THAUMETOPOEA WILKINSONI* CATERPILLARS*

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The poison hairs of the *Thaumetopoea Wilkinsoni* caterpillars produce a rash, "caterpillar dermatitis" (Fig. 1), on direct contact with the skin, described and investigated by Ziprkowski et al (1) and by others (2, 3, 4). If they penetrate the eye, the poison hairs cause a severe irritation (nodular conjunctivitis) and other pathological modifications, leading sometimes to blindness (Fig. 2). In the respiratory tract, the poison hairs cause dyspnea and chest pain.

These insects, as well as other thaumetopoidae producing similar symptoms, are not uncommon in Israel. They cause damage to forests and constitute a menace to health. The purpose of this investigation was to study the toxin of the poison hairs of these caterpillars in an attempt to find a specific antidote.

Thaumetopoea Wilkinsoni caterpillars have in addition to the usual non-poisonous hairs (macrotrichia), a special kind of poison hairs which fill small alveoli situated on the tergum of the caterpillar, on each of the last eight segments (Fig. 3). The poison hairs are 0.003–0.006mm wide and 0.15–0.50mm long. Each alveolus contains loosely adherent clusters of a large number of these poison spines. A single hair is practically invisible to the naked eye, but their bundles have a reddish-brown color and are easily distinguishable. Microscopically they appear as barbed needles, whose hooks could abrade the skin and facilitate the penetration of the poison; or possibly permit retention of the hairs within the skin after penetration. These hairs are chitinous and hollow, their central canal being filled with a refringent liquid—the poison itself (2, 3, 5, 6) (Fig. 4).

The small, barbed poison hairs are easily distinguishable from the much larger, non-poisonous macrotrichia, whose central canals are filled with pigment (Fig. 5).

In the life cycle of this insect the caterpillar instar exists during the months of October until April. About mid-February the caterpillars are fully developed and their poison hairs become very irritating to human skin. These poison hairs retain their toxic properties for many years after having been shed (1, 2, 3, 4, 7). Thus, pine forests infested by this insect constitute a menace to health all the year round, and for a long time after the disappearance of the caterpillars. They build their nests between bundles of needles and small twigs, so that they can be seen only with great difficulty. One nest usually contains about 50–100 caterpillars, but may contain as many as 500. The caterpillars shun the daylight and leave their nests only by night, in search of food. They move in a line—tail touching head—a sort of "procession", and are therefore also called "processionary caterpillars" (1, 3, 8).

The butterfly (imago) and the cocoon (pupa) have no urticating properties, but the nests and the skin (exuviae) shed by the larvae on passing into the next instar contain generally a great number of hairs capable of producing urticaria.

Although little is known of the chemical nature of the toxin of the poison hairs, it seems clear that the toxin is soluble in water, ether and diluted glycerin (1, 4, 7). The physiologic nature and localization of the toxin are only sparsely documented (2, 3, 9). Recently the results of a more systematic although still very incomplete chemical approach to the problem of urticating spines have been published (10, 11); but in this case the insect studied was the *Megalopyge Opercularis* and not a thaumetopoeid.

The aim of our work was the isolation, purification and the chemical identification of the toxin from the poison hairs of the larvae of *Thaumetopoea Wilkinsoni.*

EXPERIMENTAL

Initially we sought better solvents for the extraction of the toxin from the poison hairs. We devised a workable technic for the collection of the poison hairs from their alveoli without the use of a magnifying instrument. We then tried static and

Received for publication May 25, 1965.
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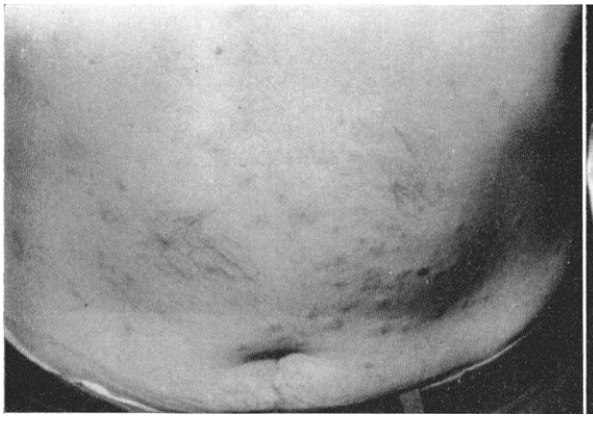

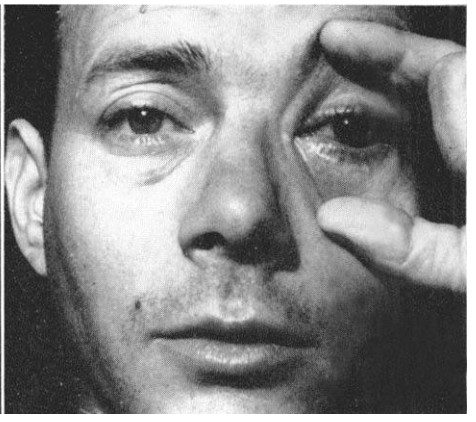

FIG. 1. Rash produced by poison hairs of Thaumetopoea Wilkinsoni caterpillars
FIG. 2. Acute irritation of eye produced by Thaumetopoea Wilkinsoni caterpillars

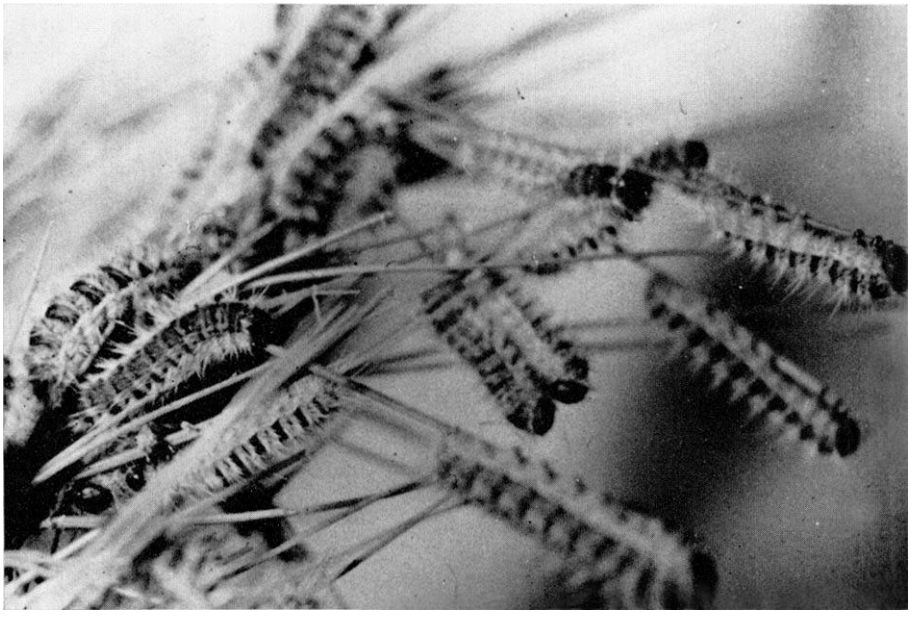

FIG. 3. Thaumetopoea Wilkinsoni caterpillars

dynamic extractions of the hairs (whole or crushed) in more than 50 different solvents, using mechanical rotators and shakers. The activity of the extracts obtained was tested by patch and scratch tests on volunteers. In order to evaluate the relative efficacy of each solvent and of each extraction procedure, it was necessary to note the rapidity and the degree of severity of the reaction obtained on the skin, and to take into account the pigmentation of the skin.

Each extract was applied for 15 minutes with a small cotton wool pad on the inner side of the forearms or the arms, and was held in place for 15 minutes with a loosely applied narrow strip of adhesive plaster. The toxin from the poison hairs does not usually produce a rash on intact skin (2, 6, 9); especially when the skin was dry, *i.e.* not perspired or artificially wetted. In order to simulate the naturally-occurring slight errosion of the skin by the poison hairs, we applied the cotton wool pads with extract after having eroded the skin very slightly with a thin glass rod. The same procedure was, of course, observed in the case of the "blank" tests. The cotton wool pad was then removed and the degree of skin reaction was estimated. This was the so-called "rapid" reaction. The test site was examined 24 hours later and the degree of the "late" reaction was noted. A

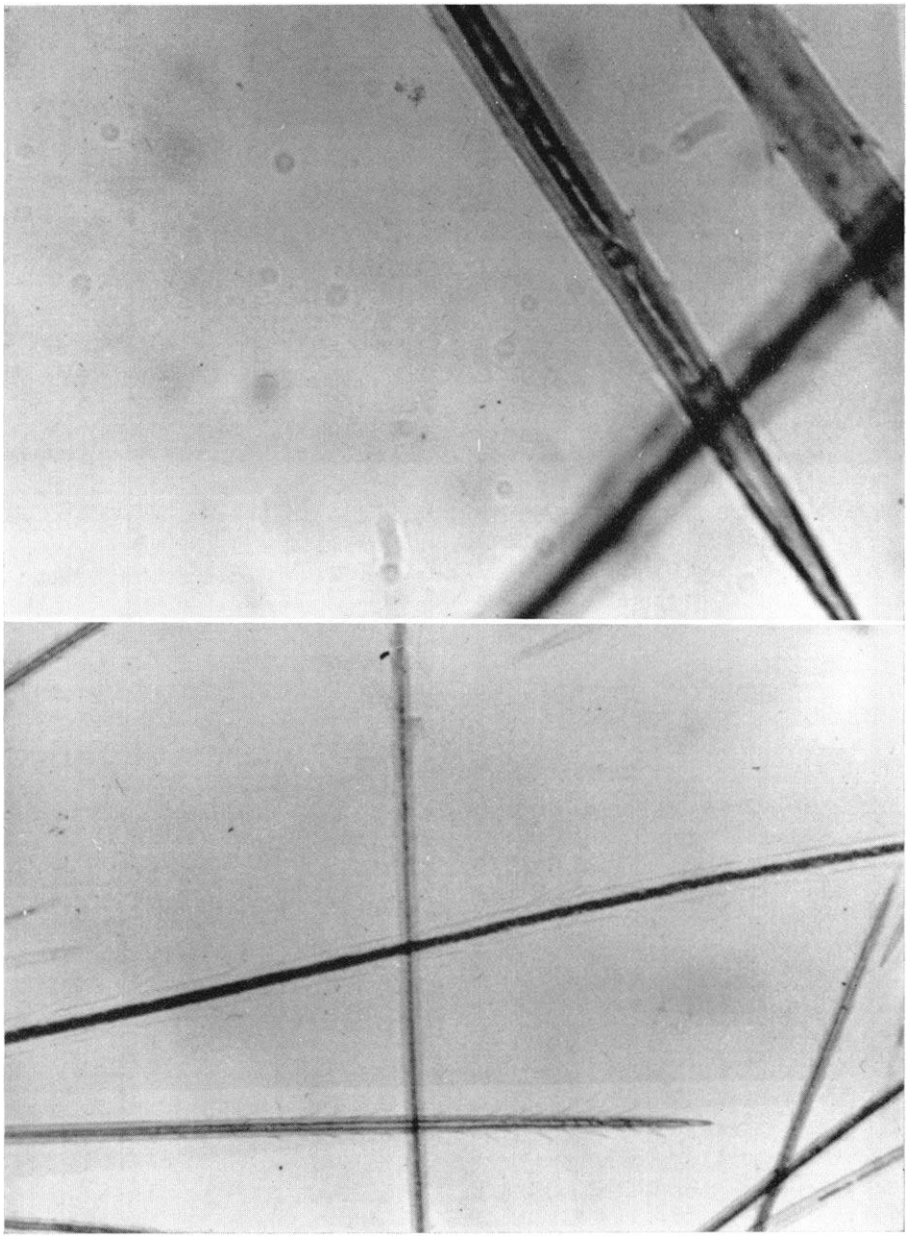

FIG. 4. Poison hairs—central canal filled with poison (× 800)
FIG. 5. Poison hairs and non-poisonous hair (much longer and filled with dark pigment) (× 400).

"blank" test, *i.e.* the application of the solvent being investigated, was performed on each volunteer.

At the beginning of our experiments the extracts were not standardized and the test results revealed only qualitative values.

To better standardize these procedures, the poison hairs were weighed before and after extraction. On the basis of loss of weight the quantity of extracted material was determined, and by relating it to the volume of solvent used, the exact concentration of the extract was established. Moreover, only those extracts were compared quantitatively which were prepared under similar conditions: same method of extraction, same duration and temperature of extraction, same solvent and

same batch of caterpillars which had previously been tested as to the toxic activity of their poison hairs.

The sites of application of the extracts on the human body were numbered in order to facilitate controls, as well as to record and compare the results.

The intensity of the skin reaction was evaluated in relation to its speed ("rapid" or "late"), as well as on the basis of a scale of intensity. This scale ranged from "no reaction" (no visible signs and no subjective complaint), to which we attributed (−), to the rapid appearance of redness and vesicles on an area surpassing that of the application, associated with very unpleasant itching and burning, lasting for at least 4 hours (6+). All the intermediate grades were given an appropriate number of plus signs.

RESULTS

On the basis of well over 1000 tests performed on more than 220 volunteers, we could establish the following facts:

a) Skin with a deeper pigmentation is generally more resistant to the toxin, giving a lesser reaction.

In order to compare the results obtained with individuals having different skin pigmentation, we established a scale of pigmentation ranging from reddish-white to brown, and each tested individual was graded accordingly. In this way we were able to compare the results obtained with the same category of toxic extracts on individuals of the same class of pigmentation; and on the basis of this experience we could compare the results obtained in individuals with different grades of pigmentation.

b) Each individual, even in the same category of skin pigmentation, reacts in a different manner to the toxin; women seem to be more susceptible to its action (6).

We avoided testing known arthropod reactors or individuals with an atopic background. Nevertheless, in a series of tests repeated on two of our co-workers, it seemed possible that sensitisation to the toxin had occurred. This phenomenon, if true, will be studied later.

c) In addition to the known solvents of the toxin, we were able to establish the following new ones: 1) petroleum ether (40–60°C); 2) carbon tetrachloride; 3) turpentine oil; 4) benzen; 5) saponin 5% (in water); 6) acetone; 7) absolute alcohol, and 8) olive oil.

d) Extraction of inner organs of the caterpillars without the integument, as well as extraction of whole caterpillars after complete removal of their poison hairs, resulted in very slightly irritating extracts, a fact not yet fully explained and probably in agreement with the observations of Fabre (3).

e) Microscopic examination of poison hairs immersed in different solvents permitted (if the solvent in question was an efficient one) the observation of a gradual dissappearance of the poison droplets from the central canal of the hairs. This made it possible to double check the efficacy and the completeness of the different extraction procedures.

f) It could be established beyond doubt that only the toxin itself contained in the poison spines is responsible for the inflammatory reaction (caterpillar dermatitis) produced on the human skin. Thus, poison hairs, after extraction and after being checked microscopically for the emptiness of their poison canal, produced no rash upon application on the human skin, whereas the extracts obtained were active in almost all instances, sometimes producing severe reactions on the skin.

g) From 100 mg of dry poison hairs we generally obtained about 22 mg of toxic substance; more complete extractions permit the assumption that 100 mg of dry poison hairs contain about 25 mg toxin in its natural form.

h) Upon heating extracts of poison hairs in different solvents at various temperatures and for various periods of time, it was apparent that the toxic substance extracted is thermolabile: after 1 hour at 42–43°C all extracts lost their toxic activity.

i) The toxin in the poison hairs seemed to be much more resistant to heat; poison hairs remained active even after being exposed for one hour to a temperature of 80°C.

j) Nearly all the concentrates obtained through evaporation at temperatures not exceeding +40°C lost a great part of their toxic activity.

The last three observations (h, i, j) indicated that the toxic substance extracted from the poison hairs in our experiments might contain proteinous matter which produced toxic symptoms on the human skin and which was denaturated by heating and drying. This was subsequently confirmed.

Extracts, concentrated through evaporation,

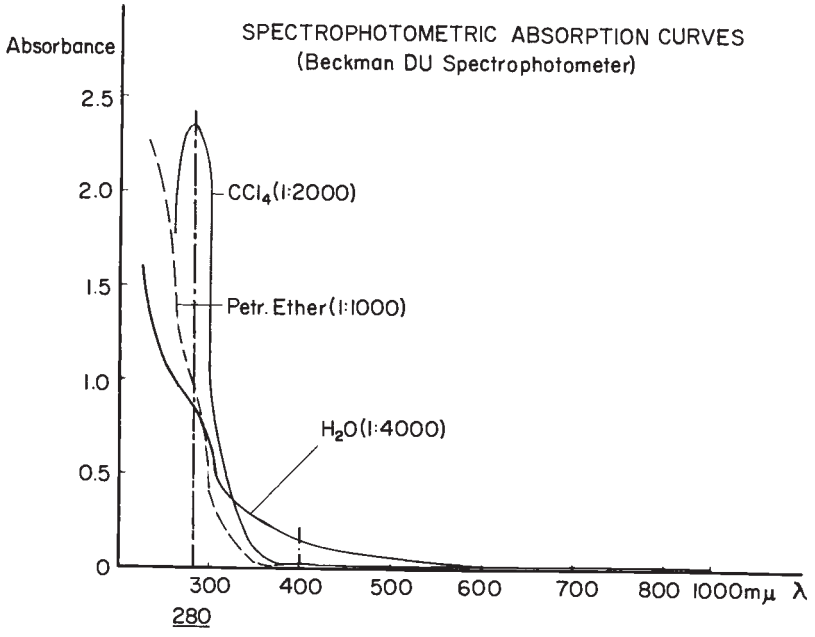

FIG. 6. Spectral absorption curves of extracts

left a residue in the form of colorless, greasy droplets, which had a characteristic "fatty" smell.

Extracts of poison hairs in water showed a pH of 6.60–6.70 at room temperature, varying a little with concentration and temperature changes.

In a previous work on caterpillar dermatitis, Ziprkowski *et al* (1) found that the toxin of the poison hairs does not produce hemolysis *in vivo*. On the basis of the present experiments we were able to demonstrate that this toxin does not produce hemolysis *in vitro*.

The presence of proteins in the toxic material extracted from poison hairs was ascertained by the following tests, which all gave positive results: the biuret reaction, the xanthoprotein reaction, the ninhydrin reaction and the reaction with trichloroacetic acid.

Paper electrophoresis for the identification of proteins showed that the toxic substances from extracts contained a great quantity of albumin, some α, β, and γ-globulins and a hitherto not identified cathodic fraction.

The spectral analysis of extracts in water, carbon tetrachloride and petroleum ether all showed an absorption maximum at 280 mμ (Fig. 6), which is characteristic for proteins and for the amino acids tyrosine and tryptophan (12). This analysis was done in the range of 200–1000 mμ with a Beckman DU Spectrophotometer. The other observed features of the absorption curves (*e.g.* a very slight maximum at 400 mμ etc.) have yet to be confirmed with more concentrated extracts.

The presence of carbohydrates (polysaccharides) could be established on the basis of positive Molisch tests. Until now we could not ascertain the presence of oligo- and monosaccharides in the toxic material extracted from poison hairs. Paper chromatography and thin layer chromatography of the toxic material extracted in various solvents also showed the presence of polysaccharide fractions.

These chromatographic analyses revealed also the existence of a great proportion of phospholipids, as well as phosphoric esters, aliphatic and aromatic amines and aliphatic acids (free or bound). By the aid of gas chromatography (Fig. 7) the following aliphatic acids were found in a more significant proportion: linoleic, linolenic, palmitic, myristic, oleic and stearic.

A lipoid fraction in the toxic substance could be demonstrated by paper electrophoresis.

Paper chromatography of extracts in various

solvents showed the presence of sulfhydryl radicals in the extracted toxic material. Estimation of these SH-radicals (according to the method of Beutler (13)), showed 1.81 mg total sulfhydryl, 0.33 mg free sulfhydryl and 1.48 mg protein-bound sulfhydryl radicals, calculated on the basis of 100 mg toxic material extracted from poison hairs.

We tried spots obtained on partition paper chromatography and on paper electrophoresis as patch testings for establishing for active principle(s) in the toxin. The results obtained thus far indicate a certain activity of the proteins, and possibly of the phospholipids, but they are not yet sufficiently conclusive to warrant publication.

Estimation of elements obtained from various extracts of the toxic material are presented in Table I.

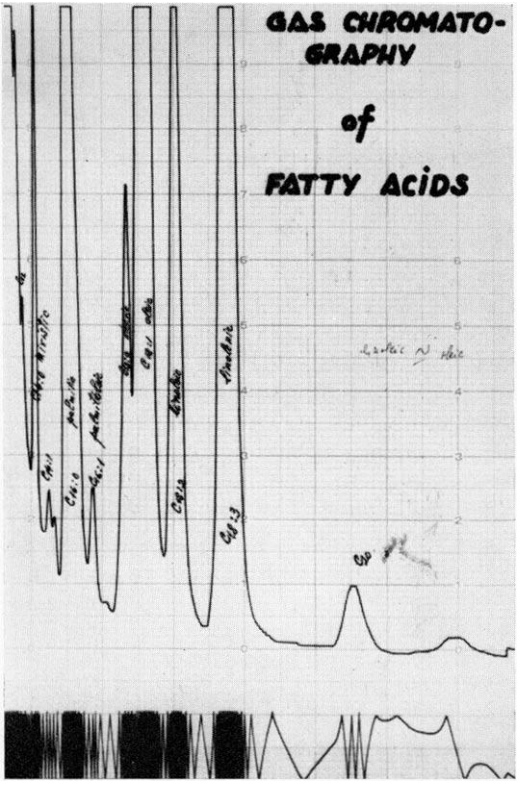

FIG. 7. Gas chromatography of aliphatic acids

SUMMARY

The results of the first portion of a systematic investigation of the poison hairs from *Thaumetopoea Wilkinsoni* caterpillars are as follows:

a) The toxic reaction ("caterpillar dermatitis") which the poison produces on the human skin is modified by the degree of pigmentation of the skin.

b) This toxic reaction is caused by the toxin contained in the poison hairs, and is not a consequence of a simple mechanical irritation.

c) The toxin does not produce hemolysis *in vitro*.

d) Eight new solvents of toxic matter from the poison hairs were found.

e) The toxin is thermolabile and contains one or more active (toxic) proteins.

f) The presence of proteins could be ascertained by spectral analysis as well as by paper electrophoresis and chemical analysis.

g) Organic analysis, paper chromatography, thin layer chromatography, gas chromatography, and paper electrophoresis revealed the presence in the extracted toxic substances of a high proportion of phospholipids, as well as polysaccharides, phosphoric esters, aliphatic and aromatic amines, lipids, aliphatic acids and sulfhydryl groups. These sulfhydryl groups and the elements K, Na, Cl and Ca were quantitated in the extracted toxin.

Appreciation is extended to Mr. J. Halperin ("Ilanot" Forest Department, Israel) for supplying us with caterpillars and nests.

This work was supported by a grant from the

TABLE 1

Estimation of elements in toxin extracts

Element	Result	Method
K	5.40 mg/100 mg extracted toxin	Flamephotometry
Na	0.12 mg/100 mg extracted toxin	Jodometric (14)
Cl	1.66 mg/100 mg extracted toxin	Mercurometric (15)
Ca	1.31 mg/100 mg extracted toxin	Corinth-blue (16)

National Council for Research and Development, Israel.

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