SOME PROPERTIES OF CUTICULAR MATERIALS (SILK, PUPAL CASE, AND WING MEMBRANE) OF *PIERIS RAPAE*

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Insect silk is considered to be a cuticular substance (Richards, 1953), and the adult wing is described as largely cuticular (Du Porte, 1959). Within the limits of relatively simple experimental techniques, some observations were made on these materials as well as on the pupal case left behind on eclosion. Particular attention was given the silk, for chemical information is said to be limited almost entirely to silk produced by the *Bombyx mori* larva (Richards, 1953).

PHYSICAL FORM AND PROPERTIES

Silk: *Pieris rapae* larvae can spin silk threads immediately after hatching, but the silk studied was that produced by the mature larva in preparation for pupation. This appears in the form of a pupal girth by which the chrysalis is suspended and a silken mat (including a button of silk at the anal end of the pupa).

The girth is approximately 23–27 microns in diameter and consists of about 9–14 individual filaments approximately 4–6 microns in diameter (vs. 11 microns observed for a monofilament isolated from a commercial silk thread; Whewell (1941) specifies a diameter of 13 microns for Italian silk and 7.8 for Canton silk). The tensile strengths of a single filament and of a whole girth were measured by suspending a cardboard cone by the filament or girth, slowly sprinkling salt into the cone until failure occurred, and weighing the final load applied. The girth (taking diameter at 25 microns) failed at a load of 7.58 grams to give a calculated tensile strength of 22,000 lb./in.^{2}; a lower value than for monofilament would be expected because several strands were involved and unequal tension would give an effective tensile strength lower than the theoretical combined strength. In accord with this, a single filament (5 microns diameter, breaking load 0.792 grams) was calculated to have a tensile strength of 58,000 lb./in.^{2} A single filament of commercial silk (11 microns, 3.476 grams) gave 52,000 lb./in.^{2}; values of 45,000 to 83,000 have been reported (Billmeyer, 1962). The above values are only approximate, of course, partly because of uncertainty in the cross-sectional area, but they do indicate that the *Pieris rapae* girth filaments are of the same order of magnitude in strength as *Bombyx mori* silk (and presumably consist of essentially the same fibroin).

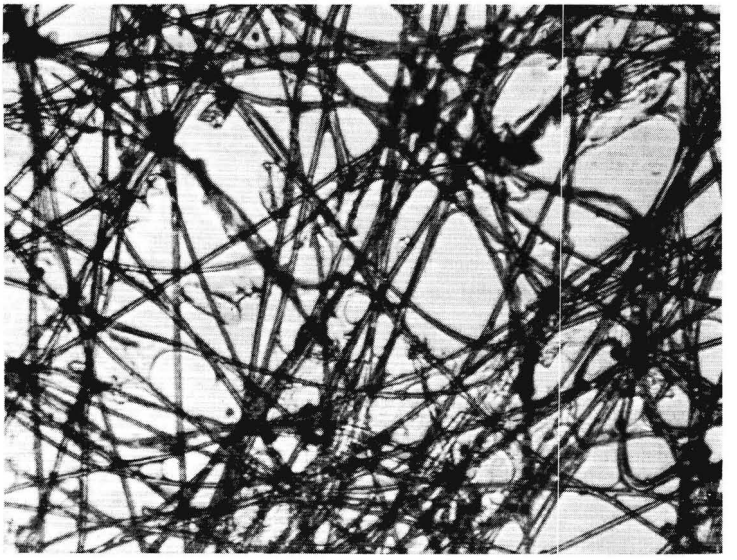

Fig. 1. Silken mat spun by larva of *Pieris rapae* (L.) prior to pupation; magnified 300 times.

Commercial silk is roughly triangular in cross section (Mark, 1951) while a *Pieris rapae* girth filament was observed to vary from about 4 to 6 microns over a distance of 5 millimeters and so could be roughly oval, triangular, or of other noncircular form in cross section.

The silken mat weighs about 0.17 milligrams (average for 10 mats) and can be peeled from gauze against which the pupa was suspended. The mat consists of fine filaments (about 3.2 microns diameter) coated and glued together with what presumably is sericin or "silk gum" (see Figure 1). Assuming a specific gravity of 1.34 (Mark (1951) gives 1.30–1.37 for raw silk) and a fiber diameter of 3.2 microns, a length of 17 yards was calculated, but this figure would be much reduced by correcting for the gum. In fact, it was very roughly estimated on the basis of Figure 1 (assumed to be representative of the whole mat) that the length is about 6 yards. In contrast to this, the *Bombyx mori* larva produces up to 4,000 yards of silk, of which up to 1,200 yards can be reeled (Whewell, 1941). It is concluded that at least part of the *Pieris rapae* silk may equal that of the silkworm in quality (at least in regard to strength); but since no cocoon is produced by *Pieris*, the quantity (length) is perhaps 0.25% of the silkworm's output.

Sericin is said to be soluble in hot water (Whewell, 1941), and it may be removed by heating with an aqueous solution of soap and ammonia

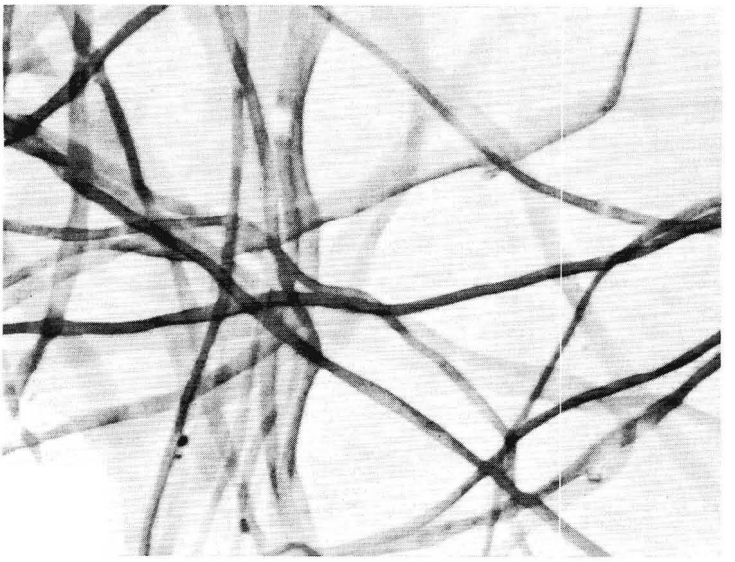

Fig. 2. Silken mat spun by *Pieris rapae* (L.) prior to pupation, shown after treatment in boiling solution of soap and ammonia; magnified 200 times.

without injuring the fibroin (Hayes, 1954). Mats (4.3 milligrams) were heated in water (1.9 ml.) at 95–100° C. for two hours, and the silk was recovered and dried (85–90° C., less than 1 inch Hg pressure, one hour) to give a matted, paperlike sheet (2.9 milligrams, 68% recovery). Raw *Bombyx mori* silk is said to consist of 11% water, 66% fibroin (which happens to agree with the 68% recovery above), 22% sericin, and 1% mineral and coloring matters (Whewell, 1941). In a more rigorous treatment, mats (4.7 milligrams) were boiled with a solution of 0.3 grams potassium stearate and 4.4 grams 28–30% aqueous ammonia in 20 ml. deionized water for 80 minutes. The visible silk was isolated, washed with water, and vacuum-dried to give a low recovery (0.4 milligrams, 9%); the remainder was solubilized or dispersed. The purified silk from another run was dried on microscope slides and stained with 0.9% aqueous Rhodamine B or Malachite Green solutions, which dyed commercial silk also. Silk, like other protein fibers, has an affinity for members of nearly all classes of dyes (Clayton, 1940) and is combined with readily by basic dyes (Hayes, 1954). Figure 2 is a photomicrograph of the Malachite Green-dyed sample. The complete removal of the gummy material is apparent.

The refractive index of *Pieris rapae* silk (mats) and commercial silk thread is approximately the same; both became almost invisible in a

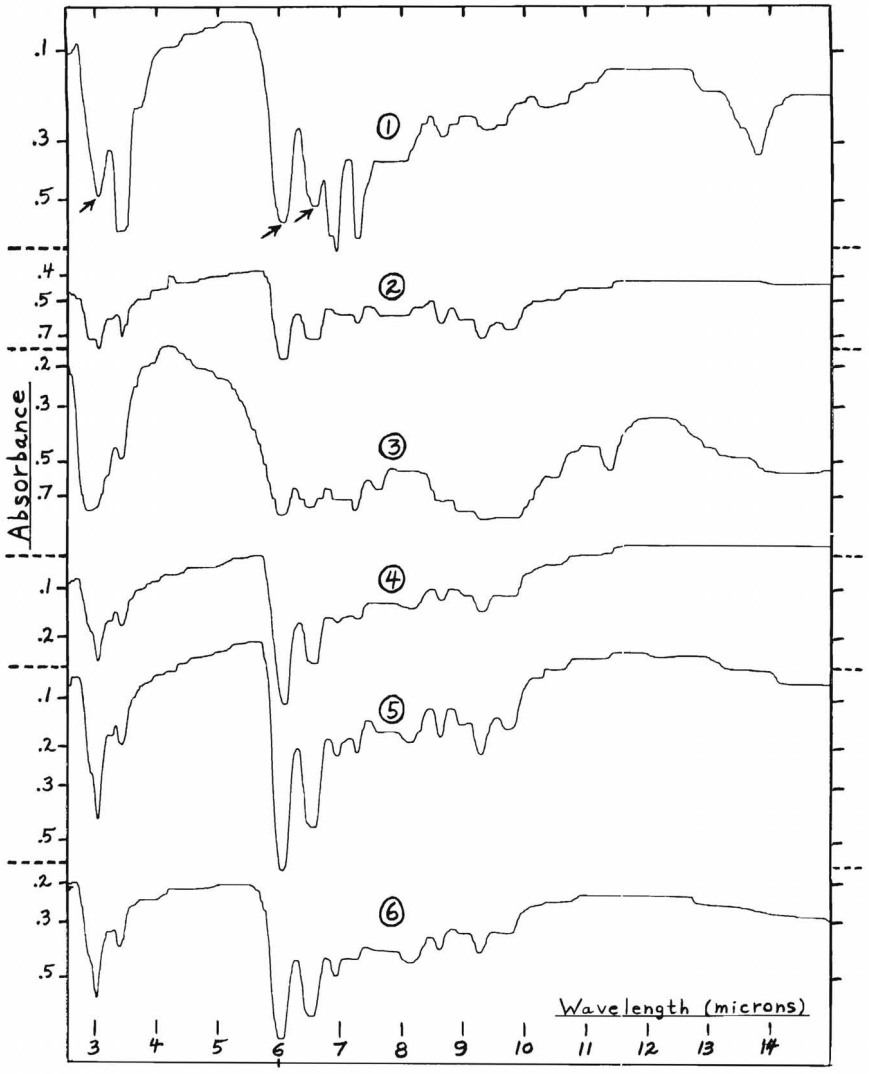

Fig. 3. Spectra obtained with an Infracord Model 137B spectrophotometer (twelve-minute scan). The pupal case and wings (descaled by rubbing) were mounted over holes in cardboards and placed in the sample beam. 1, *Pieris rapae* (L.), silken mat wetted with Nujol (Paraffin oil) between NaCl plates with Nujol between NaCl plates in the reference beam. 2, *P. rapae*, pupal case. 3, Chitin (Matheson, Coleman, and Bell, Practical Grade), through 325 mesh screen, in a KBr disc (15 milligrams chitin/gram KBr; 0.2 gram disc, about ½ inch diameter). 4, *P. rapae*, wing. 5, *Catocala cara* Gn, wing. 6, Bumblebee (*Bombus*), wing.

medium of refractive index about 1.54, which is the value reported for silk (Whewell, 1941).

Pupal Case: The case is on the order of 2.5 μ (0.1 mil) in thickness and 0.9 milligrams in weight (average of 4).

Wing: Like insect cuticle in general (Richards, 1953), the descaled wing membrane showed no strong birefringence, and gradually turned brown, but did not melt or flow, on heating in air to 360° C.

Infrared spectra (See Figure 3)

Silk: Rather good spectra were obtained on the silken mats wetted with chloroform, carbon tetrachloride, or Nujol and pressed between salt (NaCl) plates. The three bands of major interest (marked with arrows in Figure 1) are attributed to the N–H portion of the amide linkage (about 3.0 microns) and the carbonyl group (Amide I band at 6.0–6.1 microns). Also, the typical Amide II band (of uncertain origin) appears at 6.5–6.6 microns. (For a general discussion of polyamide spectra see Bellamy, 1958.) The other strong bands are due to the Nujol.

It is interesting that the fibers of the *Pieris rapae* mat were sufficiently fine (about 3 microns) to give a good spectrum, while commercial silk, whose monofilaments are about 11 microns in diameter, would not give acceptable results by the same technique.

Pupal Case: The N–H, Amide I, and Amide II bands appear in the spectra of the pupal case and of chitin. In addition, the –OH band, not shown strongly by the silk, which has relatively little hydroxyl (due to water and serine), appears at about 2.9 microns. In the case of chitin the –OH band is stronger than the N–H band, which seems to appear as a shoulder, while the pupal case shows a stronger N–H band than –OH band. This situation is consistent with the presence of considerable protein as well as chitin in the pupal case.

Wing: In the insect wing spectra of Figure 3 the –OH band at about 2.9 microns appears as a shoulder, and the N–H band at about 3.0 microns is relatively more intense than in the spectrum of the pupal case. This suggests that there is less chitin in the wing membrane than the pupal case (see nitrogen analyses below).

Infrared spectra often are used as distinctive "fingerprints" (7–15 micron region) for pure compounds, and in the present case they show the essential chemical identity of the wing membranes of a butterfly, a moth, and a bumblebee. Incidentally, *Speyeria* and *Papilio* wings gave spectra nearly identical to that of *Pieris rapae*.

Elemental analyses (Table I) and chitin content

Silk: The nitrogen content of the silken mats is comparable to that

TABLE I

ELEMENTAL ANALYSES (BY GALBRAITH LABORATORIES, KNOXVILLE, TENN.) AND CALCULATED CHITIN CONTENTS

Material	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Calculated Chitin (%)[1]
Silken mats (*Pieris rapae*)	42.87	6.40	13.88	0
Pupal cases (*Pieris rapae*)	48.63	7.46	8.72	71
	–	–	9.07[2]	67[2]
Descaled wing (*Pieris rapae*)[3]	–	–	12.75	17
Chitin ($C_8H_{13}O_5N$), calculated	47.29	6.45	6.90	–
Chitin, practical (Haynes, 1960)	–	–	6.0–6.6	–
Sericin (*Bombyx mori*) (Whewell, 1941)	42.60	5.80	16.50	0
Fibroin (*Bombyx mori*) (Whewell, 1941)	48.53	6.43	18.33	0

[1] Calculated from % nitrogen assuming two-component system of protein at 14% N and chitin at 6.6% N.

[2] Dried at 80–85° C. and less than 1 inch Hg pressure for seven hours; 7% weight loss.

[3] The wings had been allowed to dry in the air (40–60% rel. humidity) for several weeks. Water content was estimated by drying wings (8.3 milligrams) at 100° C. and less than 1 inch Hg pressure for 10 hours; the weight loss was 0%.

recorded for *Bombyx mori* sericin and fibroin though somewhat lower, probably at least partly because of the presence of moisture (said to comprise 11% of raw silk, as mentioned above).

Pieris rapae silk (mats) was dissolved in less than five minutes by 10% aqueous potassium hydroxide at 95–100° C. or in the course of one hour by 2% sodium carbonate at the same temperature. This solubility is consistent with the absence of chitin.

Pupal Case: Two nitrogen determinations were made, one on a dried sample. Assuming a two-component system (protein plus chitin) with a modest value (14%) for protein nitrogen (so as not to exaggerate chitin content) and a value (6.6%) slightly lower than theory for chitin (because reported N values for chitin are always too low, as mentioned by Richards, 1953), chitin contents of 71% (undried sample) or 67% (dried sample) were calculated. Values of 47% chitin (as % of dry cuticle) have been reported for the *Sarcophaga* puparium (Richards, 1953). The chitin is supposed to be combined with protein in the form of a complex of the materials.

As another approach to determining chitin content, pupal cases (3.5 milligrams) were heated with 2% sodium carbonate solution at 95–101° C. for three hours, recovered, and dried at 85–90° C. and less than one inch Hg pressure for one hour; the final weight was 2.3 milligrams (34% loss), and correcting this for the weight loss (6%) on drying an untreated sample under the same conditions gives 28% weight loss, presumably protein, which should be removed by this procedure (Haynes, 1960).

Similar treatment with 10% potassium hydroxide gave a corrected weight loss of 27%; treatment with 5% KOH is said to remove protein (Rudall, 1954). The same KOH treatment of practical-grade chitin resulted in a loss of 12%, and both the KOH-treated pupal cases and chitin gave a positive chitosan test (by grinding 2–10 milligrams material in a glass mortar with two drops of a solution prepared by addition of 1.2 grams iodine and 1.6 grams potassium iodide dissolved in 1.5 ml. water to 50 grams of 20% aqueous acetic acid and then by adding seven drops of 50% sulfuric acid to give a violet slurry if chitosan is present, or an orange-brown slurry in the case of untreated chitin).

Pupal cases, like chitin, were not completely dissolved by heating in 50% sodium hydroxide at 130–136° C. for four hours, and the residual matter gave a positive chitosan test. The pupal cases that had been treated with 2% sodium carbonate gave a doubtful or weak chitosan test (brownish-purple color).

In conclusion, both the nitrogen analysis and the alkaline treatments suggest a chitin content of very approximately 70% for the pupal case.

Wing: A value of 17% chitin was calculated from the nitrogen analysis (Table I). The presence of chitin was shown qualitatively by the fact that descaled wings were incompletely dissolved by 50% NaOH at 130–135° C. for four hours; the residual matter gave a positive chitosan test. A wing was dissolved almost completely by concentrated hydrochloric acid (38% HCl) after five hours at room temperature. Chitin can be dissolved by this acid (Whistler, 1953).

Note that the infrared spectra, as discussed above, are consistent with a considerably higher chitin content for the pupal case than for the wing.

Amino acids

Silk: Silken mats (6.9 milligrams) were hydrolyzed by heating with 0.48 ml. concentrated hydrochloric acid (38% HCl) at 95–99° C. for 1.5 hours. The resulting brown solution was boiled down to about 0.05 ml. and spotted approximately ⅝ inch from the shorter edge of a 3 × 4.5 inch sheet of Whatman No. 1 filter paper, which then was dipped (spotted edge down) to a depth of about ¼ inch in a layer of solvent within a closed container according to the ascending method of paper chromatography (See, for example, Lederer and Lederer, 1953). After 45–60 minutes, the chromatogram was dried in an oven at 109° C. for two to four minutes, dipped in 0.25% ninhydrin in acetone, and dried for another two minutes in the oven to develop the spots, which were encircled (and colors noted) immediately, before they began to fade. Known solutions of the amino acids in concentrated hydrochloric acid were used for comparison.

s-Collidine (saturated with water) as solvent gave excellent results; glycine (rust red; R_f 0.07), alanine (purple; R_f 0.13), and tyrosine (gray–green; R_f 0.36) were identifed in the *Pieris rapae* silk hydrolyzate and in a similar hydrolyzate of commercial silk thread by means of both the R_f values (ratio of distance traveled by spot to distance traveled by solvent front) and the distinctive colors. It is understood, of course, that identification in this manner is never positive, but in the present case there seems little reason for doubt. Serine was identified (rose-brown; R_f 0.21) in the case of both the mats and the commercial silk using phenol (saturated with water) as the solvent, while leucine (violet; R_f 0.67; pink spot appeared on ageing) was found using n-butanol (saturated with water) in the case of the mats but not the silk thread. This is explained by the fact that sericin, which is washed from raw silk, contains principally glycine, alanine, tyrosine, and leucine, while fibroin contains mainly glycine, alanine, tyrosine, and serine (Hayes, 1954).

Judging by the relative size of the spot, the proportion of leucine was much reduced in the hydrolyzate of the fibers recovered (Figure 2) when mats were boiled with soap and ammonia solution as described above. This is consistent with the anticipated concentration of leucine in the "gum" visible in Figure 1.

Pupal Case: Pupal cases were hydrolyzed and chromatographed as above. Glycine, alanine, and tyrosine were tentatively identified, while the serine spot seemed relatively weak.

Wing: Hydrolyzed wings gave the same result as the pupal cases. Thus, both chitin and protein were qualitatively identified in pupal cases and wings, and the nitrogen analyses (and alkaline treatment of the cases) give some idea of the chitin/protein ratio according to the simple two-component conception.

SUMMARY

Some observations were made on the physical and chemical properties of cuticular materials of *Pieris rapae*.

The filaments in the silken pupal girth are comparable to commercial silk (fibroin) in ultimate tensile strength, while the silken mat beneath the pupa consists of filaments heavily laden with a viscous liquid, presumably sericin, which is removed by boiling with an ammoniacal soap solution. Elemental analysis, infrared spectrum, and the presence of glycine, alanine, tyrosine, serine, and leucine in the acid hydrolyzate suggest that the mat is generally similar to raw *Bombyx mori* silk.

The presence of chitin in the pupal case left behind on eclosion was shown qualitatively by the infrared spectrum and the chitosan test, while glycine, alanine, and tyrosine were tentatively identified by paper

chromatography on the acid hydrolyzate. A chitin content of very approximately 70% was calculated from the elemental nitrogen analysis and was supported by the results of alkaline extractions supposed to remove protein.

The presence of the same amino acids was indicated for the descaled wing as for the pupal case, and again chitin was shown to be present by the infrared spectrum and by the chitosan test. However, the spectra indicated a lower level of chitin in the wing than in the pupal case, and, in accord with this, nitrogen analysis indicated very approximately 17% chitin in the wing. The infrared spectra of the wing membranes of *Pieris rapae* (and other butterflies), a moth (*Catocala*), and a bumblebee (*Bombus*) were nearly identical.

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