Table 1. Effect of sonication on protein content of yeast. Results expressed as micrograms of protein (in cells) per milliliter of suspension.

Time (min)	Sonic power setting	Protein content	
		Sonicated	Control
0			296
	0		
30		308	308
	14		
60		288	336
	0		
105		286	348

ammonium ion was determined from analyses of the amount of ammonium remaining in the medium as a function of time. The collected ammonia was assayed by the Conway microdiffusion technique and the Nessler test. Two cell suspensions were treated identically except that one was sonicated (at power setting 15) during the uptake of ammonium ion, and the other was not. In both suspensions 24 percent of the ammonium ion was taken up per hour, indicating that sonic vibration does not interfere with the normal functioning of the cell wall and membrane.

The effect of sonication at low intensity on the net protein content of the cells was investigated; the results of a typical experiment are shown in Table 1. There was small decrease in the protein content during sonication, but thereafter protein content remained constant during 45 minutes of recovery. Apparently the protein synthesizing system was unable to recover as rapidly as the systems participating in the synthesis of AIR and ureidosuccinic acid. It is noteworthy that the latter systems recovered from sonication during the period when there was no net synthesis of protein in the population. This suggests that recovery of the ability to synthesize AIR and ureidosuccinic acid does not require the synthesis of new enzymes. The rapid recovery of these systems after sonication gave no evidence of the lag to be expected if enzymes had to be resynthesized. It is unlikely that the enzymes are damaged at the low sonic powers used in these experiments; the enzyme that forms ureidosuccinic acid from aspartate and carbamyl phosphate, aspartate transcarbamylase, can be extracted in active form from *Escherichia coli* by subjecting cultures to sonication at full power for 20 minutes (*8*).

Viability tests were made to determine whether the cells recover not only the ability to synthesize ureidosuccinic acid and AIR but also the ability to continue division through many generations. Viability was tested by plating suitable dilutions of sonicated cells on nutrient agar and scoring colonies after incubation for 2 days. Before being sonicated at power setting 15, the cells were treated briefly at power setting 10, so that biosynthesis was not inhibited but cell clumps if present were broken up. The results showed that 75 percent of the cells were viable after treatment for 30 minutes at power setting 15. Since the synthesis of both ureidosuccinic acid and AIR was completely inhibited during sonication, one can conclude that inhibition occurred in cells capable of further reproduction after sonication. Sonicated cells were also observed at magnification × 1000 in a phase microscope. No lysed cells were seen, the complement of granules was normal, and buds were not knocked off mother cells; there was some indication that the vacuoles were enlarged.

What is the mechanism of inhibition by sonic treatment? The high viability of sonicated cell populations and the rapid recovery of biosynthesis after sonication indicate that DNA and the enzyme-forming systems at least, and probably the enzymes, are not inactivated by low-power sonication. If eddies form inside the cells during sonication it would be expected that the weakest bonds, such as those involved in intermacromolecular interaction, would be most easily disrupted. A loosely linked but ordered arrangement of macromolecules in the cell has been described by Frey-Wyssling (*9*). A kinetic analysis by Pollard (*10*) indicates that the enzymes of certain pathways would have to be confined to small volumes, such as membrane channels, to account for the rates of synthesis observed in the cell. The disruption of such structures may account for the inhibition of biosyntheses during sonication.

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11. Supported by PHS grant AM-03727 by the National Institute of Arthritis and Metabolic Diseases. Most of the work was done in the biophysics laboratory, Stanford University, Stanford, Calif.

9 September 1964

Adhesiveness of Spider Silk

Abstract. *Moths, by virtue of the loose scales that cover their wings and bodies, are admirably adapted to elude capture by orb-weaving spiders. Rather than sticking to the web, they may simply lose some of their scales to the viscid threads, and then fly on. Other insects, covered with detachable hairs or waxy powder, are similarly protected against entrapment. Quantitative data are presented on the adhesiveness of spider thread to insect cuticles of various kinds.*

Moths, like any insects that fly by night, profit from the relative absence of diurnal predators such as birds, yet their nocturnal excursions are fraught with other hazards. For those that fly consistently high and above the existing vegetation, the chief enemy is probably the foraging bat. Those that fly lower face danger from still another source, the spider web.

Recent work has shown that some moths are remarkably equipped for defense against bats. Special ears on the thorax enable the moths to hear the ultrasonic chirps emitted by bats in their attempts to echo-locate insect prey. Upon hearing the chirps, the moths dive downward or enter upon otherwise evasive flight, thereby eluding capture (*1*). The special way in which moths and certain other insects are adapted for escape from spider webs is the subject of this report. The study was prompted by the casual observation that moths do not necessarily get caught in a web, but may simply bounce off or fly through it.

The web of an orb-weaving spider

typically consists of a spiral of sticky silk (the so-called viscid spiral), superimposed on a framework of nonsticky supporting threads. The viscid thread derives its stickiness from tiny droplets of an adhesive fluid, spaced at regular intervals along the length of thread (Fig. 1, part *B*). On impact with the web, an insect adheres to the threads, whereupon the spider pounces on it, envelops it in silk, bites, and eventually feeds on it.

A simple experiment served to illustrate the tendency of moths to avoid entanglement in the web. Two lamps were placed at night on opposite sides of an orb (of *Argiope florida* Chamberlin and Ivie), and were then lit singly, in alternation. The purpose was to draw flying insects to one of the lamps and to follow their fate as they were subsequently lured across the web to the other lamp. Virtually all those insects, other than moths, which were observed, including primarily Coleoptera, Hemiptera, and Neuroptera, were intercepted by the web and remained trapped within it. By contrast, moths, which on that particular night consisted predominantly of Noctuidae, were detained only momentarily by the web, and usually flew off, seemingly unaffected by the encounter. However, they invariably left behind, stuck to the particular viscid threads (Fig. 1, part *E*) that bore the impact, some of the scales that ordinarily cover their wings and bodies (Fig. 1, part *F*). The scales evidently can be detached with great ease and are simply torn out by the adhesive threads when the moth strikes the web or during the brief ensuing struggle; coated with scales, the threads are no longer adhesive, and the moth is free to escape. Scale-laden patches of web—or "moth scars" as we came to call them —can commonly be seen on any but the freshest orbs, and offer mute testimony to the fact that encounters like these occur also under more natural circumstances. Obviously, moths do not always escape. Judging from our own cursory observations, smaller moths are more likely to escape than larger ones. The former simply brush against a thread or two, and fly clear through the web. Larger ones may not fit the open spaces and are hence more likely to be detained: fluttering violently, but without ever really getting firmly stuck, they "glide" along the orb, leaving behind them a glisten-

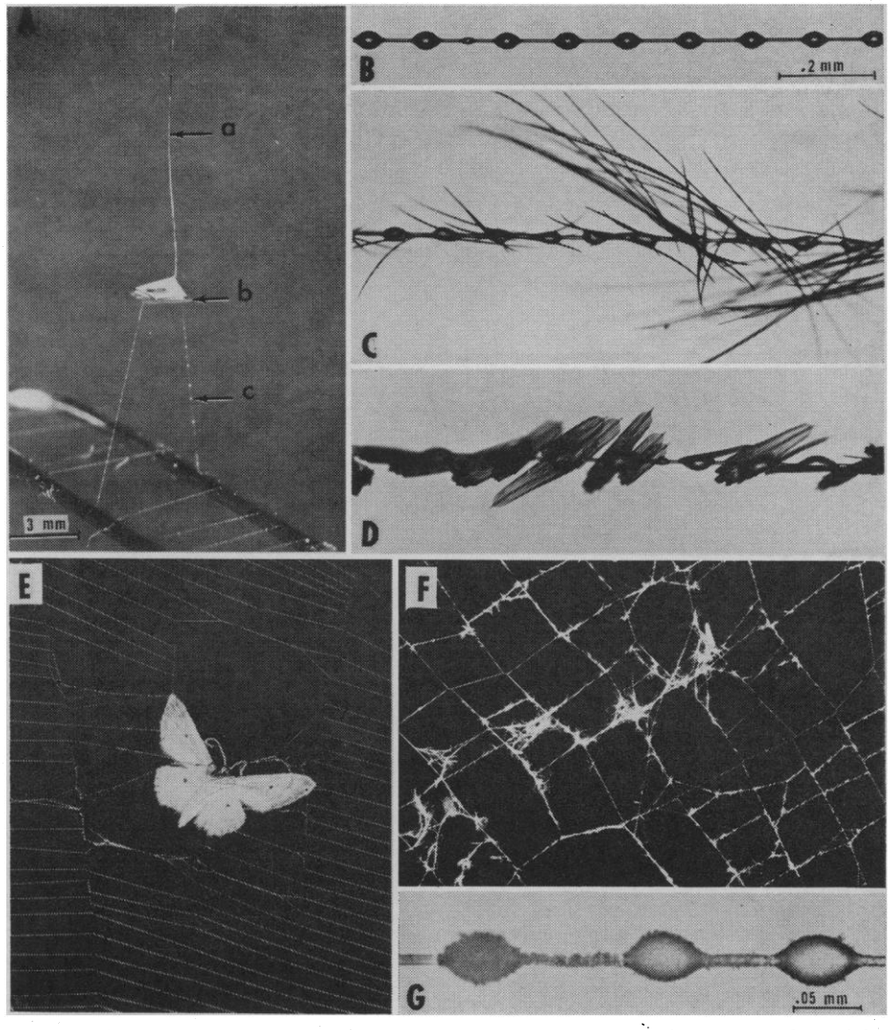

Fig. 1. *A*, Closeup of portion of apparatus shown in Fig. 2; *a*, wire hanger; *b*, cuticle; *c*, viscid spider thread. *B*, Section of viscid spider thread. The beaded appearance is due to the droplets of adhesive fluid spaced along the thread. *C*, Viscid spider thread, beset with the hairs left behind by a previously adhered piece of caddis-fly wing. *D*, Same as preceding, but with the scales of a moth. *E*, Small moth, fluttering in spider web. Note that the threads in its vicinity are already covered with scales. *F*, Scale-laden portion of spider web, where a moth had previously struggled. *G*, Small portion of a viscid thread, beset with the waxy powder from the wing of a white fly (Aleurodidae) that brushed against it.

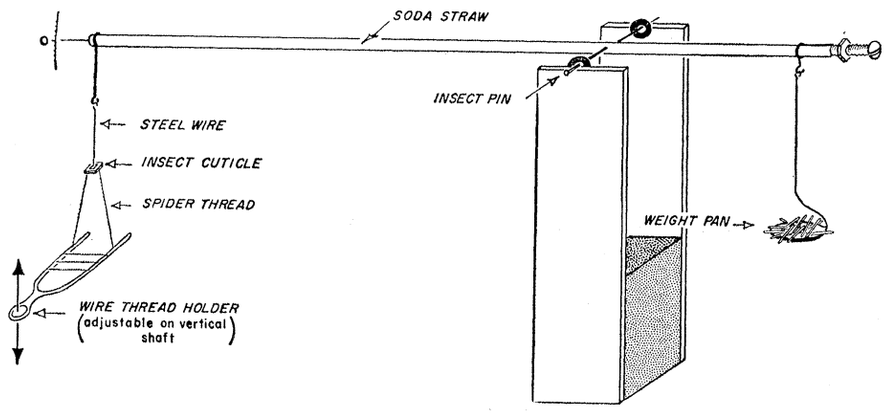

Fig. 2. Apparatus used for measuring the adhesive strength of spider silk. The vertical shaft along which the thread-holder is raised or lowered has been omitted.

Table 1. List of insects tested. The numbers correspond to those listed along the abscissa in Fig. 3. Families are given in parentheses.

1	*Pachydiplax longipennis*	(Libellulidae)
2	*Pantala flavescens*	(Libellulidae)
3	*Schistocerca alutacea*	(Acrididae)
4	*Ormenaria rufifascia*	(Flatidae)
5	*Lethocerus uhleri*	(Belostomatidae)
6	*Tabanus aar*	(Tabanidae)
7	*Sceliphron caementarium*	(Sphecidae)
8	*Hydropsyche* sp.	(Hydropsychidae)
9	*Oecetis* sp.	(Leptoceridae)
10	*Crambus mutabilis*	(Pyralidae)
11	*Euchlaena deplanaria*	(Geometridae)
12	*Logoa pyxidifera*	(Megalopygidae)
13	*Heterocampa astarte*	(Notodontidae)
14	*Felita jaculifera*	(Noctuidae)
15	*Catocala coccinata*	(Noctuidae)
16	*Danaus gilippus*	(Danaidae)
17	*Papilio palamedes*	(Papilionidae)

ing trail of scale-covered threads. The struggle may suffice for the spider to make its catch. The successful moth is the one that reverses its direction of flight promptly after impact and essentially bounces off the web, or the one that lands near the margin of the orb and glides off without delay.

In order to measure with some precision the adhesiveness of spider silk to the cuticle of moths and other insects, a crude but adequately sensitive apparatus was constructed (Fig. 1, part *A*, and Fig. 2). Previous work on spider silk had dealt with its tensile strength and other physical characteristics (*2*); nothing was known about its adhesive strength. The apparatus was essentially a modified "soda-straw balance," such as is used in some American elementary schools (*3*). One arm of the balance bore a detachable wire hanger, to the looped end of which was glued a small square (2 × 2 mm) of the particular insect cuticle to be tested. The cuticle was brought in contact with a single strand of viscid spider thread, held across the two prongs of a metal holder. Small wire weights were then added, one at a time, to the pan at the opposite arm of the balance, up to the point where the thread abruptly detached from the cuticle. During the entire procedure the arms of the balance were kept level by moving the thread-holder gradually downward along the vertical shaft that held it, so that there was compensation for the elastic stretching forced on the thread by the growing load on the pan. The load on the pan at the point of detachment—appropriately corrected to represent the counteracting load at the opposite arm—provided the basis for calculating the strength of the adhesive bond between cuticle and thread in dynes per millimeter of thread (*4*). Each piece of cuticle was tested thrice, with a different thread each time, and it was ascertained that the thread was always attached to the cuticle along a line where no thread had previously adhered. The threads all stemmed from several orbs made by a single female of the large spider *Nephila clavipes* (Linn.).

All cuticles tested were from insect wings. The species are listed by number and name in Table 1, and again by number and common name along the abscissa of Fig. 3. They comprise two categories: those whose wings are beset with scales or loose hairs (moths, butterflies, caddis-flies), and those whose wings lack such structures. The cuticles of the former group were tested both in their intact condition, and after their scales or hairs had been totally removed by stroking with a fine brush (for each species, the same piece of wing-cuticle was used for the two sets of measurements).

The results, plotted in Fig. 3, clearly show that adhesive strength is a parameter of some variability. It is nevertheless strikingly apparent that the cuticles with hairs or scales are the ones from which the thread detaches most readily; as expected, detachment involves a tearing away of the cuticular outgrowths (Fig. 1, parts *C* and *D*). If these cuticles are deprived of their outgrowths, they stick as tenaciously as do other cuticles that are normally "naked."

Interestingly, in one of the butterflies (*Papilio*, Table 1, No. 17) there was no appreciable difference in the adhesiveness to intact and "naked" cuticle, an indication that the scales of this species are relatively firmly anchored. Although this species is large and powerful, and hence undoubtedly capable of destroying small webs through fluttering, it shares with other butterflies the possession of a relatively

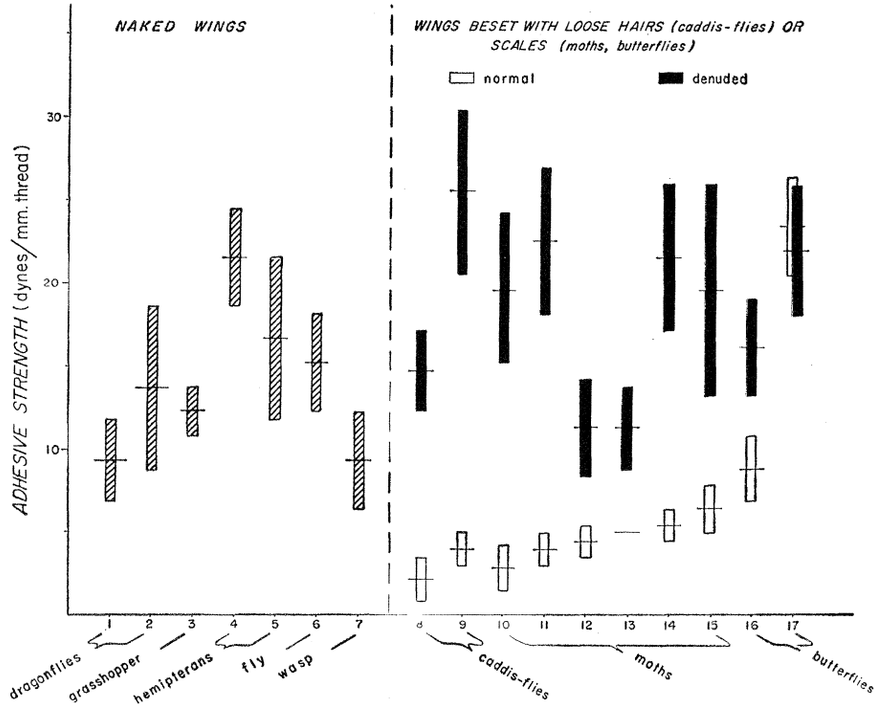

Fig. 3. Adhesive strength of spider thread to different types of insect wings. For each cuticle, the transverse line indicates the mean, and the bar, twice the standard error on each side of the mean. The species listed by number along the abscissa correspond to those formally identified in Table 1.

large wing surface and a consequently high susceptibility to entrapment. When *Papilio* was thrown by hand into large orbs of *Argiope florida*, it stuck to the webs and was caught by the spiders. Even *Danaus* (Table 1, No. 16), whose scales are more loosely attached, suffered the same fate.

There exist insects whose wings and bodies possess a flaky or powdery covering, supposedly of wax. Among them are the so-called white flies (Hemiptera, Aleurodidae). Minute in size, their wings could not be tested by our technique. However, when they were held in forceps and brushed against spider thread, they did not adhere at all. Subsequent examination of the thread showed it to be densely laden with the "waxy" powder (Fig. 1, part *G*).

Tests were also made to determine the adhesiveness of viscid thread to the cuticle of the spider itself. The two pieces of cuticle (of *Nephila clavipes*) that were tested, from the ventral opisthosoma and the patella of a leg, respectively, were shown to adhere with a strength comparable to that of "naked" insect wings (13 to 18 dyne/mm for the opisthosoma and 11 to 14 dyne/mm for the patella). Of course, a spider ordinarily restricts contact with the web to the claws at the tips of its legs, and generally clings only to nonadhesive fibers. Nevertheless, the possibility exists that the claws or tarsi, or their accessory structures, are actually nonadhesive, but this remains to be investigated.

In conclusion, it seems clear that an outer coating of detachable and partly dispensable structures—of which the scales of moths are but one example—is a distinct adaptive asset to any flying insect. It would be improper to conclude that a decreased vulnerability to capture by spiders is the sole advantage to be derived from the possession of such a coating. Certainly in the case of moths, the dense scales might also play a subtle aerodynamic role. But orb-weavers are an ever-present hazard to any insect on the wing, and their role in forcing upon their prey the evolutionary aquisition or refinement of any mechanism that reduces chances of entrapment can certainly not be dismissed.

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3. Admittedly, a more sophisticated apparatus could have been used, but this research was conceived and carried out under field conditions, and all techniques were improvised from readily available materials. We thank Professor Philip Morison (Cornell University) who some years back showed Eisner the soda-straw balance while the two were working at Educational Services Inc., Watertown, Mass.
4. This calculation involved converting the corrected load to a force in dynes, and then dividing by 2 (since the length of thread attached to cuticle was 2 mm).

5. This study was supported in part by grant AI-02908 from the National Institutes of Health. We thank R. Archbold, director, Archbold Biological Station, Lake Placid, Fla., for kindnesses while doing this work at the Station; Professors H. H. Levi (Harvard) and J. de Wilde (Wageningen) for criticisms of the manuscript. The former also identified the spiders. The insects were identified by the following specialists: W. L. Brown, Jr. (Hymenoptera), J. G. Franclemont (Hemiptera, Lepidoptera, Orthoptera, Trichoptera), J. P. Kramer (Hemiptera), L. L. Pechumen (Diptera), and M. J. Westfall (Odonata).

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Histamine: Differences in Amount Available for Release in Lungs of Guinea Pigs Susceptible and Resistant to Acute Anaphylaxis

Abstract. *Susceptibility to acute anaphylaxis in guinea pigs is related to the quantity of liberable histamine available for release in the lung. In highly susceptible Hartley animals this amount can be more than 10 times greater than in the resistant strain 2. Strain 2 and Hartley guinea pigs are equally susceptible to histamine toxicity, and their organs fix I^{131}-labeled antibody equally well.*

There has long been interest in the genetics of allergic reactions in man despite limited possibilities for experimentation. Although rats and mice are not incapable of reactivity, guinea pigs continue to be preferred for research in hypersensitivity. Recent availability of inbred, histocompatible guinea pigs (*1*) of the Sewall Wright strains 2 and 13 (*2*) now opens the way to study of the genetics of allergic reactions (*3, 4*).

Individual and strain differences in susceptibility to contact agents have been investigated by Chase (*5*). In other kinds of experiments on delayed hypersensitivity it has been reported that strain 2 is more resistant to induction of allergic encephalomyelitis than either strain 13 or Hartley guinea pigs (*4*). Concerning immediate-type reactions, studies on differences in susceptibility to anaphylactic reactions were undertaken years ago by Zinsser and Enders (*6*) who compared their susceptible guinea pigs from dealer J with resistant animals from dealer M; Zinsser and Enders used a reversed passive anaphylaxis system (rabbit antiserum against horse serum).

During studies on protracted anaphylactic shock, the finding that strain 2 guinea pigs were considerably more resistant than Hartley animals to the acute (bronchospasm) phase of anaphylactic shock (*3*) led us to try to elucidate the mechanism of the bronchospasm and the basis of the differences in susceptibility.

The possibility that the strain difference in susceptibility to acute anaphylaxis was due to differences in susceptibility to histamine was investigated by intravenous injection of histamine and use of histamine aerosols. No differences were found between strain 2 and Hartley animals either in the amount of an intravenous lethal dose of histamine or in susceptibility to histamine aerosols. The intravenous minimum lethal dose for strain 2, Hartley, and strain 13 is roughly the same as that reported previously—about 0.3 mg/kg (*7*).

The possibility that the strain differences reflected differences in the sequestering of passively administered antibody was investigated by measurement of rabbit I^{131}-labeled antibody to egg albumin in the various tissues 19 to 24 hours after intravenous injection of the labeled antibody in Hartley and strain 2 guinea pigs. No differences were discernible in the sequestration of antibody in lung, intestine, spleen, skin, liver, or muscle.

Because there might be genetic differences in the amount of histamine present in the lungs of guinea pigs, we determined by bioassay (*8*) the histamine content of tissues from the Hartley strain, strain 2, and strain 13 (Table 1). There were significant differences in the histamine contents of certain tissues. Most striking was the threefold difference in lung histamine between strain 2 (10.5 ± 4.5 μg/g) and Hartley