Preparation of Submicroscopic Spider Threads for Particle Studies

Abstract. *Many small spiders produce threads of different diameters, some of which are submicroscopic. When the threads are subjected to an environment of tobacco smoke and then examined microscopically, the invisible threads are manifested as particles in a line with no visible connection. These threads are used to support microscopic water droplets in single-particle scattering experiments.*

In 1947 H. Dessens reported a discovery which enabled him to capture, photograph, and study the microscopic haze droplets present in the atmosphere (*1*). By inducing a "small black spider" to walk about on a net of relatively coarse spider threads wound on a frame, he found that the small spider produced threads which, although invisible under the highest-power optical microscope, were manifested by captured haze droplets and dust particles. These threads were later viewed and measured by means of an electron microscope and were determined to have a minimum diameter of 0.01 μ (*2*). Using these threads, Dessens captured microscopic haze droplets ranging in diameter from 0.4 to greater than 1.6 μ and determined their size distribution (*3*). Aside from capturing haze droplets, these invisible threads can support a variety of microscopic objects upon which numerous experiments can be performed. However, Dessens' method of obtaining these threads appears to be quite complicated; a relatively simple method is presented here.

In the course of studies of the scattering properties of the aerosol we have found that: (i) many different types of spiders produce threads of both large and small diameter, the smallest being invisible under a high-power optical microscope (90× apochromat, 1.30 numerical aperture oil immersion objective, 12.5× eyepiece); (ii) these invisible threads are produced simultaneously with the coarse thread down which a spider descends in its attempt to reach a surface; and (iii) if this coarse thread is wound upon a frame in a random pattern, it will be interconnected by some of the submicroscopic threads. These findings allow one to obtain invisible spider threads without going through the complicated procedure of inducing a small spider to walk about on a net of coarse webs.

The spiders used in our experiments were found in fields, basements, and the laboratory. No attempt was made to locate a particular species of spider. Some spiders produced submicroscopic threads every time they generated a coarse thread, while others produced them only occasionally. Body lengths of the spiders from which we have obtained fine fibers varied from 0.1 cm to 0.3 cm. These submicroscopic threads are probably the same type described by Fabre in his observations of the aerial ability of the garden spider *Epeira diadema* (*4*). This spider emits an invisible thread which is caught and drawn out by the wind. When the strand is long enough the spider seizes it with his legs and is borne away by the breeze, often for a great distance.

In this report we will (i) detail the procedure used to determine whether or not a particular spider produces invisible threads; (ii) describe the frame upon which the threads are mounted to capture microscopic objects and which facilitates their examination under a high-power microscope; and (iii) indicate to tobacco-smoke researchers and others the possible utility of these threads in their studies.

A spider having an undetermined ability to produce invisible threads is placed on an object from which it eventually escapes by generating a coarse thread. A metal frame is brought into contact with this thread to which it adheres because of the thread's adhesive properties. The thread is then wound upon the frame as the spider lengthens it in its descent. Many of the threads stretched across the frame are visible to the unaided eye by scattered light. The tension of the threads can be adjusted by means of a tensioning mechanism. The frame and the threads are then subjected to an environment of tobacco smoke, after which the threads are examined under an optical microscope. If the spider has produced submicroscopic threads, the particles of tobacco smoke, about 1 μ in diameter, will be caught on the threads and will be visible through the microscope as particles in a line with no visible connection (Fig. 1). The larger webs, also about 1 μ in diameter, fail to capture the smoke particles because of aerodynamic effects. Once we have ascertained that a spider produces submicroscopic threads, it is used to furnish the threads which are employed in atmospheric aerosol experiments and other research.

The threads of a spider are examined microscopically by attaching them to a frame consisting of two rigid wires connected by solder to two pieces of brass (Fig. 2). The outside dimensions of the assembly are approximately those of a standard microscope slide with the wires comprising the long dimension of the frame. The wires are about 19 mm apart, with the span subject to extension by means of a screw-type tensioning mechanism positioned between the wires. A length of 0.03-mm copper wire is wound around and stretched between the two rigid wires in a nonoverlapping pattern. The spider's thread is also wound around these two rigid wires and a microscope cover glass is placed on the frame so that it rests upon the copper wires. The threads stretched across the top of the rigid wires can then be examined with either an oil immersion or lower power objective.

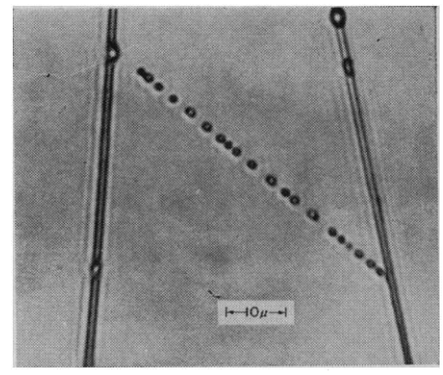

Fig. 1. Photomicrograph showing particles and droplets of pipe tobacco smoke attached to an invisible spider thread. The invisible thread is attached to two larger threads about 1 μ in diameter. The globules on the larger threads are placed there by the spider. Photomicrograph taken with a 90× oil immersion apochromat.

Since individual tobacco smoke particles and droplets are used to determine the presence of submicroscopic spider threads, it appears that techniques similar to those used by Des-

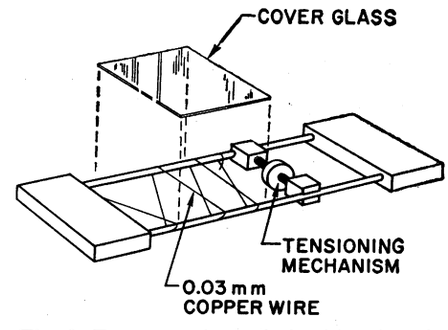

Fig. 2. Frame used to wind spider threads for microscopic studies.

sens might be employed to determine the distribution in size and concentration of tobacco smoke particles and droplets before and after inhalation and so determine the percentage of droplets of each size range trapped in the respiratory system. Thus the method of studying, by high-power optical microscopy, tobacco smoke particles supported on invisible spider threads may well be a useful addition to techniques currently in use.

Aside from their use in the capture of objects of submicron diameter, the invisible threads make it possible to perform a variety of experiments on single microscopic particles. For example, scattering experiments can be performed on a thread-supported microscopic water drop in the visible region of the spectrum without the necessity of employing levitation apparatus to support the droplet. Evidently the only scattering experiments on a single microscopic droplet are those of Egan (*5*) and Rowell (*6*), who studied the scattering of visible radiation from a microscopic oil droplet suspended by means of a Millikan oil drop apparatus. On the other hand, we have found that scattering experiments can be performed on a microscopic water droplet formed by controlling the relative humidity of the environment of a small salt crystal caught on a submicroscopic spider thread (*7*). Because the diameter of the droplet is much larger than that of the invisible supporting thread, surface tension does not cause an appreciable deformation of the spherical droplet. Further, scattering theory predicts that the radiation scattered by the invisible thread will be orders of magnitude below that scattered by, say, a droplet 1 μ in diameter. Consequently, the supporting thread will probably affect neither the shape of the droplet nor the measurement of the scattered radiation.

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Magnesium Pemoline: Failure To Affect in vivo Synthesis of Brain RNA

Abstract. *The effect of magnesium pemoline on the synthesis of brain RNA in vivo was studied. No significant effect either on the concentration of RNA or on the uptake of H^3-uridine into RNA was detected.*

There has been much interest in drugs that influence brain RNA and protein synthesis, with respect to their effects on learning and memory (*1*). Recently, Glasky and Simon reported (*2*) that magnesium pemoline (Abbott 30400; Cylert, a combination of 2-imino-5-phenyl-4-oxazolidionone and magnesium hydroxide) enhanced the activity of the RNA polymerase of brain when the drug was studied under either in vivo or in vitro conditions. A concurrent report by Plotnikoff (*3*) indicated that the drug enhanced both learning and memory in a conditioned avoidance-response situation. Although a causal relation between stimulation of RNA synthesis and an effect on learning and memory was specifically disavowed, on the basis of the data reported, it was suggested that magnesium pemoline might be used to establish such a relation (*2*).

According to Glasky and Simon, at 30 minutes and 120 minutes after the intraperitoneal injection of magnesium pemoline (20 mg/kg) the "true" RNA polymerase activity of nuclear aggregates prepared from brains of treated animals was increased. A similar stimulation of enzyme activity was reported when the drug was added in vitro to RNA polymerase preparation, but only after the drug was made soluble with dimethyl sulfoxide. No attempt was made to measure directly the effect of the drug on synthesis of RNA in the brain.

If the reported effects on the activity of brain RNA polymerase are meaningful, an effect of the drug on synthesis of RNA in brain in vivo ought to be readily demonstrable. We have measured the effect of pemoline on the concentration of RNA in the brain and also on the amount of incorporation, into brain RNA, of injected H^3-uridine. The test rats (100 to 150 g, Sprague-Dawley strain) were given magnesium pemoline intraperitoneally in doses of 5, 10, 20, or 40 mg/kg (*4*). This range of doses brackets the dose previously used, 20 mg/kg (*2*). Animals in the control group were injected with water, or with $MgCl_2$ at the same concentration as pemoline; $MgCl_2$ had no apparent effect. One hour later, H^3-uridine (0.5 ml aqueous solution; 2.5 μmole, 100 μc/μmole) was injected into the tail veins of the rats. One hour after this injection (2 hours after the drug) the rats were guillotined; the brains were removed in the cold, and the RNA content and the specific activities were determined. Weighed samples of the tissues (0.3 to 0.5 g, one cerebral hemisphere) were homogenized briefly in 4 ml of ice-cold water to which was added 6 ml of 10 percent trichloroacetic acid (TCA) as soon after homogenization as possible. The precipitate was recovered by centrifugation and was washed six times with cold 5 percent TCA. After the last wash no radioactivity remained in the supernatant fraction. The final precipitate was drained well, 2 ml of 1*M* NaOH was added, and the samples were incubated for 16 hours at 37°C. These samples were then acidified to precipitate DNA and protein, and the supernatant fraction was assayed for orcinol-reactive material and material absorbing at 260 mμ, as well as for radioactivity. The values obtained from the two methods of measuring the concentration of RNA-nucleotide were

Table 1. Effect of pemoline on the concentration of RNA in the brain and the specific activity of RNA in both control and pemoline-treated rats. Each value represents the mean value for four animals ± the standard error of the mean. When experimental mean values are compared to control mean values for each experiment, the differences are not statistically significant according to Student's *t*-test.

Pemoline (mg/kg)	Experiment	Brain RNA (μg/g)		Specific activity of RNA (count/min per microgram)	
		Control	Pemoline-treated	Control	Pemoline-treated
5	A	1654 ± 76	1705 ± 103	1.19 ± 0.09	0.98 ± 0.22
10	A	1654 ± 76	1578 ± 154	1.19 ± .09	1.16 ± .28
10	B	1601 ± 63	1584 ± 51	1.29 ± .10	1.50 ± .17
20	C	1684 ± 83	1660 ± 23	1.98 ± .20	1.70 ± .15
20	D	1752 ± 32	1515 ± 70	1.42 ± .07	1.38 ± .11
40	B	1601 ± 63	1561 ± 66	1.29 ± .10	1.22 ± .10