observed in this herd. The a/- pigs were the most frequent type for which PSS susceptibility could not be predicted. However, in seven of the eight litters in which PSS-susceptible and PSS-free a/- pigs were both present, the red blood cells from the PSS-free pigs reacted slightly more quickly and strongly with one of the Ha reagents than did those from the susceptible type. The difference in degree of hemolysis was slight, and the other Ha reagent did not show any difference. It is possible that additional Ha reagents would show a greater difference between genetically different a/- types, and it may be possible to produce reagents which react with red blood cells from one of the types and not the other. Similarly, a reagent might be prepared in such a way as to distinguish the c/- PSS-susceptible type from the c/- PSS-free type; the red blood cells from the PSS-free types reacted more quickly and completely with the Hc reagents than did those from the susceptible pig. If this difference were to prove consistent and other reagents were so prepared as to clearly distinguish between all H-system genotypes, it might be possible to accurately predict PSS susceptibility by blood typing for H-system factors.

In addition to effects of the H system on productivity and reproduction, the relation between H system and PSS is important because PSS is frequently associated with pale, soft, exudative pork (*8*). It has been suggested that the basic cause of PSS and of undesirable pork is a difference in permeability of muscle fibers. Research on H blood types has not included studies of H factors on muscle membranes (*4–6*).

PSS is also a model in pigs for a similar syndrome in humans, the malignant or fulminant hyperthermia syndrome (*9*), and it is also a possible model for the sudden infant death syndrome.

Although there have been no previous reports of association between red blood cell antigens and stress susceptibility in any species, the association between antigens in the M system of blood groups in sheep and high versus low levels of red blood cell potassium (*10*) is an example of another profound physiological effect associated with the red cell antigenic type, which also affects productivity and reproductive performance (*11*).

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Dimethyl Disulfide: An Attractant Pheromone in Hamster Vaginal Secretion

Abstract. *Dimethyl disulfide, isolated from estrous hamster vaginal secretion and identified by gas chromatography–mass spectrometry, is an attractant for male hamsters.*

That olfactory pheromones are involved in the reproductive behavior of many animals now seems to be established, but the identification of the active chemical compound or compounds for mammalian species has proceeded more slowly than for invertebrate species (*1*). In the laboratory or under seminatural conditions, many mammals do not rely exclusively on olfaction for the initiation or maintenance of normal sex behavior. However, the male golden hamster is particularly dependent on its vomeronasal and olfactory systems since their deafferentation regularly interferes with normal responses to an estrous female (*2*). The estrous female hamster produces copious amounts of vaginal secretion in synchrony with behavioral receptivity (*3*). The odor of this material has a number of different effects on male behavior, such as decreasing aggression (*4*) and flank gland marking (*5*) and increasing the number of inappropriate mounts attempted with surrogates (*6*). Independent of its ability to increase mounting, this odor elicits approach and intense investigatory behavior by both sexually experienced and inexperienced male hamsters (*7*). Because of the uniformity of the behavior pattern of approaching, sniffing, and digging, we decided to use this pattern as the basis of an assay to identify the attractant pheromone in hamster vaginal secretion.

The vaginal secretions were collected on filter paper during the evening of day 1 of the females' estrous cycles and stored at −20°C (*8*). The male behavioral assays were conducted in plastic cages (42 by 21 by 20 cm) with solid plastic covers. Two odor ports, each a cluster of five small holes (6 mm inside diameter), were drilled through the cage bottom 30 cm apart. These ports were covered by a fine wire mesh. Glass jars (30 ml) containing odorants could be firmly fixed beneath each port by a perforated screw cap attachment, which allowed test or control odors to diffuse from the jars, through the ports and the 2-cm layer of Sanicell bedding, and into the cage. A typical behavioral assay began with a preliminary 3-minute period in which both jars contained a suitable control odor, to which no test animal ever responded. Then followed a test period during which one of the jars, selected randomly, contained the odor to be evaluated. A positive response was recorded if the male approached the area over one of the odor ports and began to sniff and dig through the bedding in an attempt to gain access to the jar. The latency and duration (both sniffing and digging time) were recorded. One minute after the beginning of a response the test jar was removed and further response time at the test port was accumulated for an additional minute. In the absence of any positive response the test was terminated after 7 minutes.

Prior to being used in the behavioral assays, each male was given a 7-minute sex behavior test with a receptive female. Approximately 95 percent of the males were observed to mount and intromit on this single test and were retained in the colony. The response of these animals to the odor of vaginal secretion (from three females) was then measured in a plastic test cage. This substance attracted all of the animals. Occasional animals that spontaneously dug at the ports in the absence of odor stimuli were removed from the colony. Thus all males used to evaluate chemical samples were known to respond sexually to receptive females, were strongly attracted

to the odors of vaginal secretion, and did not dig spontaneously. All behavioral tests were conducted in the male colony room 2 hours into the dark phase under dim illumination and spaced so that at least 48 hours elapsed between successive tests on each animal. After an average of 2 weeks of testing, an animal was removed from the test cage and returned to its home cage for a 1-month rest period. For purposes of monitoring the chemical isolation, a sample was considered behaviorally active if two out of three male hamsters responded with at least 20 seconds of sniffing and digging at the test port while ignoring the control port.

Volatile compounds were isolated from the vaginal secretions by adsorption on Tenax porous polymer resin (*9*). The pieces of filter paper with vaginal secretion were placed in water (6 ml per 50 papers) at room temperature. For 5 hours nitrogen gas (10 cm^3/min) was passed through the flask containing these materials and then through a condenser maintained at 10°C and finally through a tube containing the resin at 0°C. The water used to wet the filter paper served to disperse the secretions and to establish thermal contact. The tubes containing adsorbed volatiles were stored at –20°C. These volatiles were active in the behavioral monitoring assay described above, whereas the residual water and filter papers were inactive.

The adsorbed material was transferred to a gas chromatograph (GC) column by placing the resin tube in the carrier gas line and heating it to 200°C for 4 minutes while the first 10-cm section of the column was cooled to –78°C (*10*). After transfer the resin tube and Dry Ice were removed and a normal GC procedure was initiated. Fractions of this volatile material were trapped from the column exit in tubes containing Tenax. Behavioral assays indicated that two of these fractions were active (Fig. 1). After further purification on other GC liquid phases (SF-96 and diethylene glycol succinate), followed by behavioral assay, two apparently active compounds were isolated and identified, by comparison of their mass spectra and GC retention times with those of authentic samples, as dimethyl disulfide (CH_3SSCH_3) in the first active fraction and dimethyl trisulfide (CH_3SSSCH_3) in the second fraction (*11*). By means of calibrated GC peak areas, we calculated that an average of 5 ng of the disulfide and 2 ng of the trisulfide were isolated from each estrous female hamster at each collection (*12*).

In order to compare the behaviors elicited by the authentic sulfides with those elicited by either the natural secretion or the active fractions, a concentration series of each sulfide was made up in squalane and assayed in the usual manner. Table 1 shows this comparison for dimethyl disulfide. The latency and duration scores elicited by this compound are within a factor of 2 of those obtained with either the unprocessed secretion or the first active fraction. Based on the total amounts of disulfide measured in the latter two samples, it appears that the authentic material is from 20 to 40 percent less active than either of these sources. A similar decrement in activity is apparent in the number of animals responding to a particular odor source. Since dimethyl disulfide alone does not seem to account for all the activity observed in the natural samples, there may be other compounds contributing to the activity of the vaginal secretion. The strong activity of fraction 1 suggests that it may contain such compounds. These conclusions should be tempered by several considerations. The presence of different vehicles (vaginal secretion, water, or squalane) in the various samples may differentially affect the vapor concentration of attractant. Consequently, when samples with different vehicles are compared, it is possible that the stimulus levels given in Table 1, although accurately measured by analytical GC, may not be directly proportional to the amounts actually reaching the animal. The behavioral assay does not distinguish negative from null responses; therefore, only positive responses can be interpreted. The number of positive responses during test periods was significantly greater ($P \leq .002$) than those during the preliminary 3-minute periods according to the binomial test (*13*). Three animals dug at the control port during exposure to the two highest intensities of authentic disulfide, a result suggesting that these stimuli were so strong that the animals could not accurately localize their source (Table 1).

Dimethyl trisulfide seems not to account for the activity of the second active fraction, since authentic dimethyl trisulfide is not active in the range of 50 ng to 10 μg. The thermal conversion of trisulfides to

Table 1. Quantitative comparison of the latency and duration, in seconds, and the number of animals responding with the approaching, sniffing, and digging behavior pattern to various attractants containing the indicated amounts of dimethyl disulfide. The control odor for the vaginal secretion and volatile fraction 1 was water. The control odor and diluent for dimethyl disulfide was squalane. The same 12 males were used throughout. T, test jar; C, control jar.

Attractant	Amount of dimethyl disulfide (ng)	Mean latency ± S.E.M.		Mean duration ± S.E.M.		Positive responses (No.)	
		T	C	T	C	T	C
Vaginal secretion	15	123 ± 15		58 ± 1		12	0
Volatile fraction 1	55	144 ± 31		37 ± 9		12	0
Dimethyl disulfide	128	189 ± 33	90 ± 0	65 ± 16	5 ± 5	8	1
	56	203 ± 59	55 ± 25	23 ± 10	10 ± 7	5	2
	22	204 ± 40		51 ± 16		8	0
	2	294 ± 48		26 ± 11		5	0

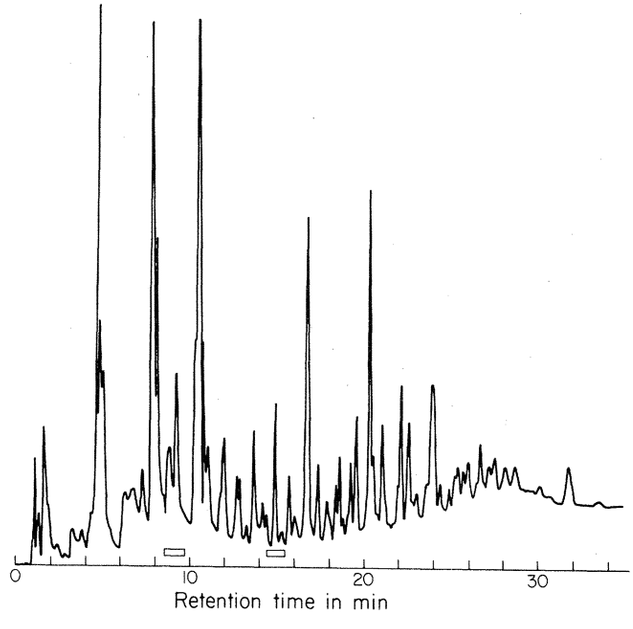

Fig. 1. Gas chromatogram of the volatile compounds from female hamster vaginal secretion. The bars under the chromatogram indicate the two fractions active in the behavioral assay. The column temperature was programmed from 25° to 200°C at 8°C per minute (*10*).

disulfides is well known (*14*), and it is likely that the activity of this fraction is due to a small amount of dimethyl disulfide formed from the trisulfide. The possibility remains, however, that there is an unidentified active compound in this fraction.

We conclude that dimethyl disulfide is present in the estrous hamster vaginal secretion. In addition, this compound, when present at stimulus intensities in the range of those to be expected from a receptive female, elicits approaching, digging, and sniffing behaviors similar to those elicited by the natural secretion. Consequently this compound is an active attractant and can be considered the likely factor in the normal attraction of the male hamster to the female. Other assay procedures will be required to determine whether this compound or some unidentified substance subserves the other behavioral responses evoked by the vaginal secretion.

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11. Mass spectra were obtained with a DuPont 21-492 double focusing mass spectrometer operating at a resolution of 1000, an ionization potential of 140 volts, and an ion accelerating potential of 1800 volts. The spectrometer scanned at 2 seconds per decade from m/e 600 to m/e 20. A new scan was initiated every 9 seconds. The data were collected on a VG 2040 data handling system. Gas chromatography peaks were identified by plots of total ion current or of selected ion currents against scan number and by the Mülheim algorithm [D. Henneberg, K. Casper, E. Ziegler, *Chromatographia* **5**, 209 (1972)] or the VG "mass max" algorithm. For each compound three mass spectra were obtained as the peak emerged from the GC. In each case, the four most intense ions matched the m/e and relative intensity observed for authentic dimethyl disulfide and dimethyl trisulfide, respectively. We obtained the spectra of the authentic compounds under experimental conditions identical with those used to obtain the spectra of the unknowns. The spectra of the compounds from the vaginal secretion also agree with those reported in the "Eight Peak Index of Mass Spectra," (Mass Spectrometry Data Centre, AWRE Aldermaston, England, ed. 2, 1974). Finally, the probability based matching algorithm of McLafferty was used for a computer search of a file of 35,700 mass spectra [F. W. McLafferty, R. H. Hertel, R. D. Villwock, *Org. Mass Spectrom.* **9**, 690 (1974)]. The highest confidence indices were given by dimethyl disulfide and dimethyl trisulfide. The major ions observed in the unknown spectrum identified as that of dimethyl disulfide are: m/e (intensity, percent) 94 (100), 79 (75), 45 (57), and 47 (21). The ions from the unknown spectrum identified as that of dimethyl trisulfide are: 126 (100), 79 (54), 45 (45), and 80 (39).
12. Additional major components (0.5 to 30 μg per female) have also been identified from the whole volatile fraction. These include ethanol, 1-propanol, 1-butanol, 1-hexanol, and ethyl butyrate. Authentic samples of these compounds, at the appropriate intensities, proved inactive. In addition, a number of common odorants not present in the natural secretion have been examined in the usual male behavioral assay. For example, oleic and linoleic acids were inactive in milligram amounts whereas similar quantities of butyric acid and amyl acetate each elicited responses of less than 5 seconds' duration. Food odors were also inactive.
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Coordinated Activities of Middle-Ear and Laryngeal Muscles in Echolocating Bats

Abstract. *The middle-ear muscles and laryngeal muscles of the little brown bat (*Myotis lucifugus*) are highly developed. When the bat emits orientation sounds, action potentials of middle-ear muscles appear approximately 3 milliseconds after those of the laryngeal muscles; this activity of middle-ear muscles attenuates the vocal self-stimulation and improves the performance of the echolocation system. When an acoustic stimulus is delivered, both types of muscles contract; action potentials of the laryngeal muscles appear approximately 3 milliseconds after those of the middle-ear muscles. These two groups of muscles are apparently activated in a coordinated manner not only by the nerve impulses from the vocalization center, but also by those from the auditory system.*

In the echolocating bat, the cricothyroid muscle and middle-ear muscles (MEM's) are enormously hypertrophied. In man, the inferior laryngeal nerve, innervating all extrinsic laryngeal muscles except for the cricothyroid muscle, is essential for speech (*1*). In the bat, the superior laryngeal nerve innervating the cricothyroid muscle is indispensable for the emission of orientation sounds. The bat's laryngeal mechanism for the production of sound is different from that of man (*2*). Unlike man, the echolocating bat has large MEM's which appear to be powerful in controlling the transmission of sound energy across the ossicular chain. The contraction-relaxation time of the MEM's is of very short duration. The tetanus fusion frequency of the stapedius muscle ranges between 260 and 320 sec^{-1} (*3*). When the bat emits a short frequency-modulated (FM) orientation signal, the MEM's start to contract 6 to 8 msec before vocalization and usually contract maximally during vocalization. Then, these muscles relax within 2 to 8 msec after vocalization. These muscles are specialized for selective attenuation of the vocal self-stimulation, which can theoretically improve echo detection (*3, 4*). The vocal MEM activity—that is, the MEM activity synchronized with vocalization—indicates that the MEM's and laryngeal muscles (LM's) are activated in a coordinated manner by the nerve impulses originating from the vocalization center. During further experiments on the vocal MEM activity in the little brown bat (*Myotis lucifugus*) (*3*), we found that not only the MEM's but also the LM's responded to acoustic stimuli. These two groups of muscles are apparently activated by nerve impulses from the auditory system. We describe here the coordinated activities of the MEM's and LM's that appear when the bat vocalizes and when it receives acoustic stimuli.

Eighteen *M. lucifugus* were studied. With the animal under ether anesthesia, the flat head of a nail 1.8 cm long was mounted against the dorsal surface of the skull of each animal with glue and dental cement. The animal was placed ventral side up in a body-sized oblong box made of wire mesh. Its head was then held motionless by locking the shank of the nail into a metal rod with a set screw. Both the MEM's and LM's were then exposed. An indifferent electrode (silver wire) was placed on the subcutaneous tissue, and recording electrodes (sharpened tungsten wires) were inserted into the MEM's and cricothyroid muscle or inferior laryngeal