Firing frequency of the spike discharges recorded from tarsal chemosensory hairs of *Phormia*, following stimulation with NaCl solutions, was taken into account in evaluating hair responsiveness. 7- to 10-day-old adult blowflies, *Phormia regina*, were used. The insects were reared in our laboratory at 24 °C, 75% relative humidity, and fed with granulated sucrose and water. The chosen specimens were deprived of food 24 h before the experiments. Electrical resistance measurements were performed on D_5 tarsal hairs according to a method previously described by Stürckow[6]. The tip-recording procedure[7] was adopted for recording hair spike activity. The stimulating-recording micropipette was filled with 1 M NaCl. Spike-firing frequency was measured in the tonic phase of discharge for a 1 sec period starting 200 msec after the onset of the stimulation. Almost all the spikes comprising the discharges were of the same size and shape, only a few spikes being of smaller amplitude. Only the larger spikes were taken into account. As shown in the table, some hairs failed to respond to stimulation with 1 M NaCl (inoperative hairs). Their electrical resistance was significantly higher than that of the hairs in which NaCl stimulation evoked spike-firing activity (operative hairs). A regression line has been calculated by relating electrical resistance to spike-firing frequency in the operative hairs. This regression line showed that the lower the hair electrical resistance, the higher the spike-firing frequency. In fact, the correlation coefficient was equal to 0.645 and significantly $\neq 0$ according to Student's t test ($p < 0.001$ on 86 experiments). Failure to respond to chemical stimulation by a given number of chemosensilla has already been observed in labellar and wing chemoreceptor hairs of *Phormia*[8,9]. In agreement with the results of the present study on tarsal hairs, the electrical resistance of the inoperative wing hairs proved to be higher than that of the operative ones[9]. Here the mucopolysaccharide layer at the hair tips may be the important modulating factor, as indicated by previous experiments in which the hair tips were cut off[4,5]. It is consequently likely that, when hair electrical resistance exceeds a threshold level (or, in other words, when fluxes of stimulants from the external environment to the chemosensory dendrites are reduced below a given rate), the concentration of the stimulants at the chemoreceptive sites is too low to operate the chemoreceptor unit. Furthermore, our data on operative hairs demonstrate that, below threshold value, hair electrical resistance varies in a given range, and its variations influence hair responsiveness. It is very likely that, before reducing stimulant fluxes and consequently stimulant concentration below a critical non-operative level, an increase in hair electrical resistance can simply cause a reduction in spike-firing frequency, as may be deduced from the regression line of spike-firing frequency versus hair electrical resistance indicated previously.

In conclusion, our data directly demonstrate that a relationship between hair electrical resistance and responsiveness does exist in the chemosensilla of *Phormia*. Hair electrical resistance measurement is a very simple and quick procedure that can be widely adopted in chemosensitivity studies, chiefly when chemosensory hair responsiveness has to be tested in large populations of flies, or when responsiveness variations in a single chemosensillum are to be monitored for a given period of time. Electrical resistance measurements in fact require only very short periods of contact between hair tips and test solutions, thus reducing adaptation phenomena and allowing measurements to be repeated several times on the same hair without influencing its bioelectrical features.

Number of operative and inoperative tarsal D_5 hairs of *Phormia regina* Meig. and their electrical resistance when tested with 1 M NaCl (mean value ± SE)

	Number of hairs	Electrical resistance (MΩ)
Operative hairs	86 (74.24%)	26.02 ± 0.11
Inoperative hairs	32 (25.76%)	100.90 ± 9.85

Electrical resistance of the operative hairs differs significantly from that of the inoperative ones (Student's 't' test, $p < 0.001$). Number of experiments = 118.

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Antifreeze agents in the body fluid of winter active insects and spiders[1]

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Summary. Insects and spiders which are active at subzero temperatures on snow in winter are found to be protected against internal freezing by antifreeze agents present in their body fluid. The body fluid has a melting point of about -1 °C, but the antifreeze agents prevent growth of ice crystals at temperatures down to -6 to -7 °C.

One of the most peculiar features of insects and spiders which are active on snow in the winter is their ability to perform complex biological activity at low temperatures. The snow scorpionfly *Boreus westwoodi* and the spider *Bolyphantes index* perform activities such as mating, web construction and feeding at temperatures below the melting point of their body fluid[2,3], and thus, they appear to be active in a supercooled state. Because of these extreme physical conditions and the permanent and intimate contact between the animals and external ice crystals, they

Thermal data of the snow scorpionfly *Boreus westwoodi* and the spider *Bolyphantes index*

Species	Melting point	Point of crystal growth	Range of hysteresis	Supercooling point
Boreus westwoodi	−1.2±0.4 (2)	−6.4±1.1 (2)	5.4±1.6 (2)	−8.6±1.6 (10)
Bolyphantes index	−1.4±0.2 (5)	−6.9±0.9 (5)	5.2±0.9 (5)	−15.2 (24)**

Temperature in °C; mean values± SD. Number of specimens given in parenthesis. ** Taken from Hågvar[3].

seem to face a great problem in avoiding lethal internal freezing, brought about by spontaneous nucleation or by seeding of ice through their cuticle. We have found that winter active *B. westwoodi* and *B. index* are protected against internal freezing by the presence of antifreeze agents in their body fluid, which cause a thermal hysteresis between the freezing-melting curves, and thus prevent growth of ice crystals within the temperature range where these animals are normally active.

Antifreeze agents have previously been found in the blood of polar fishes living at water temperatures of −1.7 °C, about 1 °C below the melting point of their body fluid[4,5]. The antifreeze agents give rise to a thermal hysteresis of the freezing-melting curves, implying that small ice crystals present in a plasma sample will not start growing until the sample is cooled to about 1.5 °C below the melting point. This will effectively prevent internal freezing in the fishes within their natural temperature range. Antifreeze agents have also been found in the body fluids of certain hibernating insect larvae[6], spiders[7] and molluscs[8,9]. The antifreeze agents in insect larvae and spiders give rise to a thermal hysteresis of about 5 °C and 2 °C, respectively, but the adaptive significance of these antifreeze agents is not established.

Active *B. westwoodi* and *B. index* were collected from the surface of snow in the vicinity of Trondheim in March and April 1979. Samples of haemolymph were obtained by puncturing the animals and sucking the exuding haemolymph into a thin glass capillary. The melting point and the temperature at which ice crystals began to grow in the haemolymph was determined by using a Clifton nl-osmometer, where the temperature of 50-nl samples could be controlled within ± 0.02 °C while observing the samples through a microscope. The temperature at which the last ice crystal disappeared when a frozen sample was heated slowly was taken as the melting point. Presence of antifreeze agents was tested by cooling a sample containing a tiny ice crystal at a rate of about 0.7 °C per min and observing the size of the ice crystal. The presence of antifreeze agents in the sample would counteract growth of the ice crystal down to a temperature where the sample suddenly becomes opaque due to rapid propagation of ice all over the sample. This temperature was taken as the temperature of ice crystal growth. The supercooling point of intact animals was determined by cooling the animals at a rate of about 1 °C per min until they froze spontaneously. The temperature of the animals was measured by using a copper constantan thermocouple, which was kept in close contact with the surface of the animals, and connected to a Leeds & Northrup Speedomax recorder. The initiation of freezing was indicated by the rapid temperature-increase due to the liberation of heat of fusion of water.

The results, as given in the table, reveal that in the haemolymph of both *B. westwoodi* and *B. index* there was a difference of 5–6 °C between the melting points and the temperatures of ice growth, indicating that the body fluid of both these species contain antifreeze agents. In both species, growth of ice crystals was prevented down to −6 to −7 °C, indicating that the antifreeze agents are able to protect the animals against internal freezing within their natural activity range, which is reported to go down to −2 °C for *B. westwoodi*[2] and to −5.5 °C for *B. index*[3].

The antifreeze agents previously found in fish and insects have been shown to be proteins or glycoproteins[4–6]. In haemolymph from *B. index* the antifreeze effect was still present following heating of the haemolymph to +70 °C but disappeared at +80 °C. This might indicate that the antifreeze agents of this species also contain proteinous components, but no firm conclusion can be drawn until further studies have been carried out.

Antifreeze agents causing thermal hysteresis seem to be uncommon in insects[10], in that hibernating insects seem much more frequently to accumulate polyhydric alcohols (polyols). Following accumulation of polyols, the melting point of the body fluid of many hibernating insects is lowered to a level corresponding to the temperature of ice growth in the haemolymph of *B. westwoodi* and *B. index*[11–13]. This will prevent internal freezing just as effectively as the antifreeze agents. However, no winter active insects or spiders have been reported to accumulate polyols in the winter, and accumulation of polyols appears to be restricted to animals hibernating in a passive state, often involving a diapause. As pointed out by Duman[10], the tremendous increase in osmotic pressure resulting from high polyol concentrations might require considerable physiological and biochemical compensation. A factor which might have particular importance is the viscosity of the haemolymph. The viscosity of water at −2 °C is about twice the value at +20 °C, and a solution of glycerol with a melting point of −6 °C will have a considerably higher viscosity[14]. Winter active insects are likely to have a substantially higher metabolism at low temperatures than insects which hibernate in a passive state. This might require a correspondingly higher circulatory activity at low temperatures, and the energy needed to maintain an adequate circulation will probably increase proportionately to the viscosity of the haemolymph. By using antifreeze agents rather than polyols for protection against internal freezing the insects might avoid the circulatory problems associated with a highly viscous haemolymph.

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