role of each cell of these synaptic networks in generating organotypic bioelectric discharges as a model of CNS activity.

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Freezing Resistance in Polar Fishes

Abstract. *Arctic and antarctic fishes, living in contact with sea ice at — 1.9°C, have plasma equilibrium freezing points near — 1.2°C which are dependent on salt concentrations. These supercooled fishes have plasma protein concentrations much higher than other polar animals have, and the proteins impede ice propagation at temperatures down to — 2°C. Plasma protein concentration increases as environmental water temperature decreases.*

Polar fishes living near shore in −1.9°C ice-covered sea water are exposed to conditions that would normally cause death by freezing in nonacclimated fishes. Fishes in the Labrador fjords were the first to be investigated for the presence of antifreeze in their blood (*1, 2*). I now compare freezing resistance in arctic and antarctic fishes and seek to illuminate the freeze-resistant properties of their blood.

Eleginus gracilis and *Myoxocephalus scorpioides*, two arctic species living in −1.8° and −1.4°C ice-covered sea water, respectively, were caught by a hook and line at a depth of 2 m near Nunivak Island, Alaska. Two antarctic shallow-water fishes (*Notothenia corriceps* and *Notothenia gibberifrons*) were caught in 2°C sea water near shore at Anvers Island, Antarctica. Two antarctic hemoglobin-free icefishes (*Chaenocephalus aceratus* and *Pseudochaenichthys georgianus*) were caught in deeper 2°C sea waters off Anvers Island, Antarctica.

Blood samples were usually taken from the posterior end of the caudal vein or by heart puncture. Measurements of colloid osmotic pressure were made by a stretch dialyzing membrane technique (*3*). The anticoagulant heparin was used for most samples, and its small contribution to the pressure was subtracted. Total plasma protein concentration was determined by a micro-Kjeldahl method (*4*). Plasma melting points, freezing points, temperatures of initial ice propagation, and rates of ice propagation were determined by a field technique modified from Scholander *et al.* (*1*). Modifications included a viewing port on the side of a copper box insulated with Styrofoam. Adjustment of the bath temperature was accomplished using heating wires connected to a variable transformer. With zero heat input, bath temperature remained constant at −4°C. Ice crystals were observed with a 20-power microscope. A 5-mm^3 plasma sample was frozen with "Spra-Freeze" (Laboratory Supplies Co.); then melting and freezing points were determined by observing the last small upward-floating ice crystal.

The melting point, determined to within 0.01°C, was the temperature at which the last small upward-floating crystal began to blur. The freezing point, measured with equal precision, was the temperature at which the edges of this last crystal began to sharpen. In NaCl solutions the melting and freezing points show a disparity of approximately 0.01°C when this apparatus is used (see Table 1). On the other hand, this disparity can be several times greater in a concentrated protein solution. Moreover, one must be careful to distinguish the equilibrium freezing point from the temperature of initial ice propagation in plasma. The former is 0.01° to 0.09°C below the equilibrium melting point, whereas the latter can be 1.00°C below the equilibrium melting point (Table 1).

Studies of ice growth in the plasmas of polar animals necessitated a trichotomy of ice propagative characteristics (Table 1). In the control group of animals, birds and mammals, ice propagation in plasma began at temperatures just below the equilibrium freezing point and the propagation rate was linearly dependent on how far the bath temperature was below this point. Ice growth occurred by bulk freezing and took the form of feathery plumes advancing through the plasma solution. This freezing behavior was also evident in solutions of NaCl, but in these macromolecule-free solutions ice began to propagate at the equilibrium freezing point. A second group of animals, polar bottom fishes which live supercooled at −1.8°C but never contact sea ice or anchor ice, showed a slightly larger disparity between the equilibrium freezing point and the temperature of initial ice propagation. Growing ice crystals took a dendritic form in this second group also. A third group, the nearshore fishes that live in contact with sea ice at −1.8° to −1.9°C, had strikingly different ice propagative behavior in their plasmas. When the bath temperature was dropped slightly below the plasma equilibrium freezing point in these fishes (noted by the sharpened ice crystal edge), a small amount of crystal growth occurred but then suddenly stopped. Upon further lowering of bath temperature (from 0.8°

to 1.2°C below the freezing point), a temperature was reached at which sharp, monoclinic spears shot through the plasma. This was the temperature of initial ice propagation.

The rates of ice propagation in 5-mm³ plasma samples of the three groups of polar animals illustrate further the freezing resistance present in the shallow-water fishes (Fig. 1). Rates were measured in the apparatus previously described by timing the linear progress of ice needles along a 10-mm microscope reticle (100 divisions) while maintaining constant bath temperature. The polar birds and mammals exhibited a gradual increase in ice propagation rate just below their equilibrium freezing points. These plasmas displayed freezing behavior most like simple NaCl solutions in which feathered-type ice propagation began at the equilibrium freezing point. The second group, the antarctic deep-bottom fishes (*Chaenocephalus aceratus*), showed a slightly greater increase in rate with bath temperature with ice growth occurring 0.3°C below the freezing point. The third group, the antarctic shallow-water fishes (*Notothenia coriiceps*) living in contact with sea ice, exhibited a 0.9°C disparity between their equilibrium freezing point and the temperature of initial ice propagation. Furthermore, below this latter temperature, propagation rates of monoclinic ice spears increased capriciously with just slight lowering of bath temperature. Thus, these shallow-water fishes are much more resistant to freezing due to their inhibition of ice propagation between −1.18° and −2.09°C, an inhibition that is absent in other polar animals.

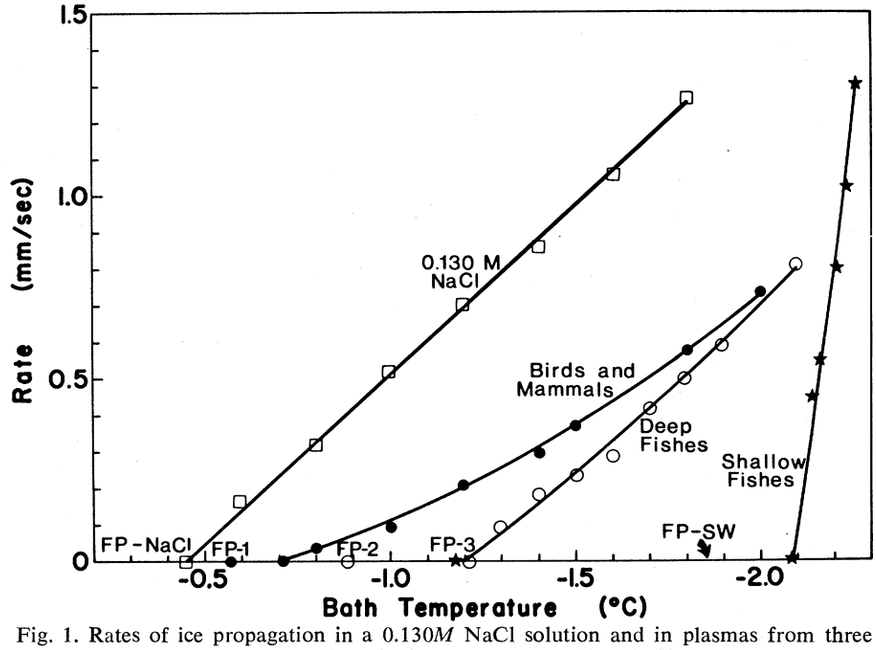

Fig. 1. Rates of ice propagation in a 0.130*M* NaCl solution and in plasmas from three groups of polar animals at various bath temperatures. The NaCl (open squares) has a freezing point (indicated by FP-NaCl) equal to its temperature of initial ice propagation (−0.45°C). The control animals (group 1), birds and mammals (solid circles), maintain a constant body temperature above ambient and exhibit freezing behavior most like that of the NaCl solution. The deep-water antarctic fishes (group 2, open circles) live supercooled but without contact with sea ice. The shallow-water antarctic fishes (group 3, stars), live supercooled in contact with sea ice. Freezing points for plasmas from groups 1, 2, and 3 are indicated by FP-1, FP-2, and FP-3. The freezing point for sea water is indicated by FP-SW.

The exact mechanism by which ice growth is impeded in ice-seeded samples of supercooled plasma remains unknown, although speculations by Umminger, Smith, DeVries, and others suggest that hydroxyl-rich compounds inhibit growing surfaces of ice crystals in the serums of supercooled fishes (*5–7*). A recent and excellent chemical investigation by DeVries indicates that serum glycoproteins with *cis*-hydroxyl groups on the galactose residues are the

Table 1. Resistance to freezing in plasmas of polar fishes. The resistance due to protein impedance of ice propagation equals the difference between the equilibrium freezing point and the temperature of initial ice propagation. All values represent means ± standard deviations.

Animal (species and number of samples)	Equilibrium melting point (°C)	Equilibrium freezing point (°C)	Temperature of initial ice propagation (°C)	Colloid osmotic pressure (cm-H_2O)	Protein concentration (g/liter)
Group 1 (controls: birds, mammals, and NaCl)					
Adélie penguin (*Pygoscelis adeliae*, 3)	−0.55 ± 0.010	−0.57 ± 0.006	−0.75 ± 0.025	20.6 ± 2.9	40 ± 6
Giant petrel (*Macronectes giganteus*, 2)	−0.56 ± 0.000	−0.57 ± 0.007	−0.71 ± 0.007	10.0 ± 1.4	28 ± 3
Spotted seal (*Phoca vitulina*, 2)	−0.55 ± 0.014	−0.58 ± 0.007	−0.62 ± 0.021	28.5 ± 2.1	
Man (*Homo sapiens*, 3)	−0.54 ± 0.010	−0.55 ± 0.006	−0.66 ± 0.006	35.7 ± 2.6	71 ± 7
0.130*M* NaCl (4)	−0.44 ± 0.005	−0.45 ± 0.003	−0.45 ± 0.005	0.0	0
Group 2 (deep-bottom fishes)					
Icefish I (*Chaenocephalus aceratus*, 11)	−0.84 ± 0.006	−0.89 ± 0.008	−1.36 ± 0.086	29.4 ± 12	60 ± 9
Icefish II (*Pseudochaenichthys georgianus*, 1)	−0.84	−0.85	−1.06	4.1	28
Group 3 (shallow-water fishes)					
Antarctic notothenid I (*Notothenia coriiceps*, 11)					
1) Caught in 2°C sea water	−0.87 ± 0.014	−0.92 ± 0.022	−1.84 ± 0.125	79.9 ± 6.8	68 ± 4
2) Acclimated to −1.8°C for 23 days	−1.10 ± 0.020	−1.18 ± 0.019	−2.09 ± 0.057	102 ± 5.7	79 ± 3
Antarctic notothenid II (*Notothenia gibberifrons*, 2)					
1) Caught in 2°C sea water	−0.88 ± 0.000	−0.91 ± 0.007	−1.73 ± 0.064	70.8 ± 4.9	61 ± 5
2) Acclimated to −1.8°C for 20 days	−1.14 ± 0.007	−1.23 ± 0.007	−2.14 ± 0.024	98.7 ± 2.3	76 ± 2
Arctic cod (*Eleginus gracilis*, 14)					
1) Acclimated to 5°C for 4 days	−1.12 ± 0.016	−1.21 ± 0.010	−1.87 ± 0.052	175 ± 9.3	
2) Caught in −1.8°C sea water	−1.18 ± 0.013	−1.25 ± 0.019	−2.16 ± 0.043	214 ± 12	
Arctic sculpin* (*Myoxocephalus scorpioides*, 3)	−1.11 ± 0.006	−1.16 ± 0.010	−2.03 ± 0.010	128 ± 2.5	

* Caught in −1.4°C sea water.

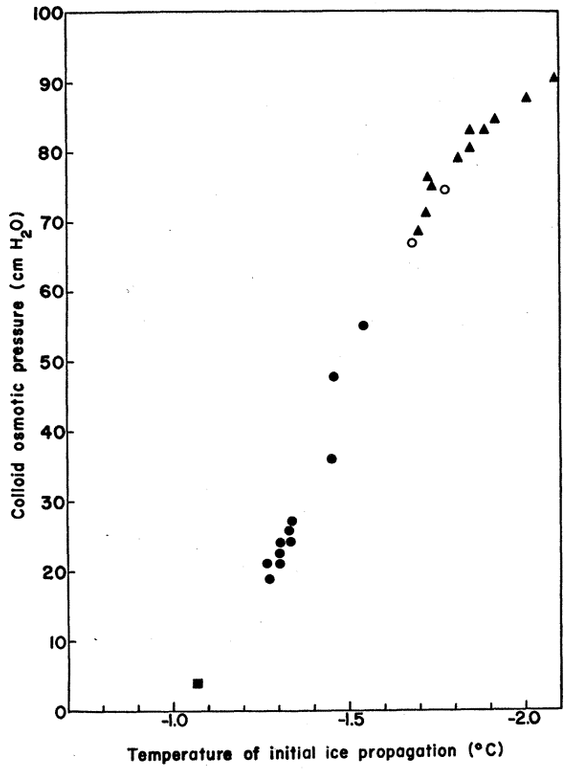

Fig. 2. Correlation of temperature of initial ice propagation to colloid osmotic pressure in antarctic deep-water fishes: *Pseudochaenichthys georgianus* (solid square at bottom), *Chaenocephalus aceratus* (solid circles), and antarctic shallow-water fishes: *Notothenia gibberifrons* (open circles) and *Notothenia corliceps* (triangles). All fishes were caught in 2°C sea water.

active agents which inhibit ice crystal growth in antarctic fishes (*6*).

Equilibrium freezing measurements (actually melting points) have demonstrated that arctic and antarctic fishes living in contact with sea ice are supercooled (*2*, *5*, *8*). The earlier freezing points determined by DeVries and collaborators (*7*, *9*) using a rate-measuring device are probably measurements of the temperature of initial ice propagation and not true equilibrium freezing point values. Consequently, although such measurements assist in identifying the antifreeze components of serum, such an apparatus fails to elucidate the actual freezing behavior. In his more recent study DeVries equates freezing point with temperature of ice crystal growth (*6*). This temperature, however, probably corresponds to the temperature of initial ice propagation as listed in this report (Table 1). The true equilibrium freezing point, identified by a sharpening ice crystal edge, is less than 0.1°C below the equilibrium melting point and considerably above the temperature of initial ice propagation (that is, the temperature of ice crystal growth).

The antifreeze components which impede ice growth at temperatures above −2°C in the antarctic nototheniids are in the protein fraction of the plasma. In order to separate the microsolute fraction from the macromolecular phase, three plasma samples from −1.8°C acclimated (23 days) *Notothenia corriceps* were ultrafiltrated in a modified centrifuge tube with a dialysis membrane as described by Scholander and Maggert (*8*). Care was taken to maintain original concentrations in each fraction. The colloid-free microsolute fraction had a slightly higher freezing point (−1.09°C) than the unaltered plasma (−1.18°C) but this microsolute fraction exhibited none of the antifreeze properties that prevent ice propagation between the freezing point and the temperature of initial ice propagation. The macromolecular fraction had a freezing point of −1.10°C and contained all the ice-inhibiting components that afford these fishes the additional 0.9°C protection below their freezing point. Similar experiments on *Notothenia gibberifrons* gave the same results. These findings verify those made earlier by Scholander and Maggert on arctic fishes (*8*) and support the thorough chemical analyses conducted by DeVries (*6*).

When the antarctic fishes caught in 2°C sea water were examined, a good correlation was found between the temperature of initial ice propagation and the colloid osmotic pressure (Fig. 2). Figure 2 also indicates the spread of plasma protein concentrations (reflected in the colloid osmotic pressure data) even within one species.

Acclimation studies suggest that there is a seasonal change in concentration of these colloidal particles, although the variation seems more pronounced in the arctic than the antarctic shallow-water fishes (Table 1). Acclimation periods are given in Table 1. The antarctic nototheniids, which experience a temperature drop of 4°C from summer to winter, exhibited a significant increase in protein concentration and associated decrease in temperature of initial ice propagation. Similar results, but greater in amplitude, were found in the arctic cod. Perhaps this is a result of their exposure to a greater drop in summer to winter temperature (usually about 12°C near Nunivak Island, Alaska). Considering the 11.3 cm-H_2O colloid osmotic pressure of a temperate-water cod (*10*), the 214 cm-H_2O mean value for the arctic cod acclimated to −1.8°C is astonishing. Certainly this high colloidal concentration protects these supercooled fishes from freeze damage. It is also interesting to note that even the freshwater arctic char (*Salvelinus alpinus*) when acclimated to 3°C from 2°C in a period of 2 weeks, displayed a decrease in mean colloid osmotic pressure from 92.5 ± 13 cm-H_2O to 26.6 ± 2.3 cm-H_2O (*11*).

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