THE FREEZING RESISTANCE OF ANTARCTIC FISH: I. SERUM COMPOSITION AND ITS RELATION TO FREEZING RESISTANCE

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ABSTRACT. In polar regions the blood of teleost fish may be supercooled for all or part of the year. The supercooled state is stable and does not endanger survival of the fish unless freezing is initiated by contact with ice. Serum from nine species of Antarctic fish was analysed to determine which component, if any, would aid survival in this circumstance. Two sub-tropical species were studied for comparative purposes. Antarctic fish serum was found to be a more concentrated solution than temperate fish serum and unusually high concentrations of serum reducing sugar occurred in most Antarctic species. In the two species which were studied both in summer and winter, the concentrations of most serum constituents increased in winter, but the increases were insufficient to lower the serum freezing point to that of sea-water. It is unlikely that inorganic serum constituents contribute significantly to the freezing resistance of a supercooled fish in contact with ice, but serum reducing sugar and possibly protein and glycoprotein appear to protect the blood from freezing, probably by preventing or retarding the nucleation and propagation of ice in the blood. A comparison of serum composition and freezing resistance showed that a knowledge of serum composition alone was insufficient to predict the survival time of a supercooled fish in contact with ice, but was possibly sufficient to predict whether or not the blood of the fish would freeze.

POLAR fish may be supercooled for all or part of the year and, while supercooled, may be in danger of freezing if they are "seeded" by contact with ice. These fish, in order to survive, must either avoid contact with ice or else be able to resist the formation of ice in their bodies if contact with ice does occur. Both alternatives occur in Arctic fish; deep-water species avoid contact with ice and shallow-water species possess a marked freezing resistance (Scholander and others, 1957).

The degree to which a fish may be supercooled depends on the osmolarity of its body fluids. The blood osmolarity of teleost fish generally increases as the environmental temperature falls (Scholander and others, 1957; Woodhead and Woodhead, 1959, 1965; Eliassen and others, 1960; Pearcy, 1961; Gordon and others, 1962; Leivestad, 1965; Umminger, 1967, 1968, 1969*a*, *b*, *c*, *d*) and, at temperatures approaching the freezing point of sea-water (−1·8° C approximately), the increase is usually insufficient to prevent the fish from being supercooled. Boreal and Arctic fish are supercooled in winter by 0·25–0·85° C and vary considerably in their resistance to freezing (Scholander and others, 1957; Gordon and others, 1962; Leivestad, 1965; Woodhead and Woodhead, 1965). The solutes ("antifreeze") responsible for the increased blood osmolarity of these fish in winter have not been completely identified and the reasons for the varying resistance to death of supercooled fish in contact with ice remain obscure.

When blood passes through the gills of a fish, it is cooled to the temperature of the surrounding water (Carey and Teal, 1966; Lyman, 1968), whereas other body fluids are warmed by metabolic heat to an equilibrium temperature slightly above that of the surrounding water. The low blood temperature of a supercooled fish and the risk that the blood may be "seeded" through the thin gill epithelium by ice crystals in the water are good reasons for beginning a study of freezing resistance by investigating the composition and properties of the blood.

The work described here was carried out at Signy Island, South Orkney Islands, and at South Georgia. This paper presents the results of a comparative chemical analysis of fish serum. A preliminary account of some of the results has been published (Smith, 1970). Two species of sub-tropical fish were also studied for comparison with the Antarctic species. This study was undertaken as a result of the observation by I. Everson and R. Ralph (unpublished results) that supercooled *Notothenia neglecta* could, in marked contrast to other *Notothenia* species, survive contact with ice for a period of several hours.

METHODS AND MATERIALS

Localities, times of year and sea temperatures

The localities and times of year at which the various species of fish were caught are given in Table I.

Signy Island lies south of the mean northern pack-ice limit (Mackintosh, 1946). In Borge Bay,

TABLE I. LOCALITIES AND TIMES OF YEAR AT WHICH FISH WERE CAUGHT

SIGNY ISLAND. Borge Bay (lat. 60°43′S., long. 45°38′W.)

Summer (December–March)	*Winter* (July–October)
Notothenia neglecta Nybelin	*N. neglecta* Nybelin
N. rossii marmorata Fischer	*N. rossii marmorata* Fischer
N. gibberifrons Lönnberg	*Trematomus newnesi* Boulenger
Chaenocephalus aceratus (Lönnberg)	
Parachaenichthys charcoti (Vaillant)	
T. bernacchii Boulenger	

SOUTH GEORGIA. King Edward Point (lat. 54°17′S., long. 36°30′W.)
Prince Olav Harbour (lat. 54°04′S., long. 30°09′W.)

Early summer (November)

N. neglecta Nybelin

N. rossii marmorata Fisher

C. aceratus (Lönnberg)

P. georgianus (Fischer)

Muraenolepis microps Lönnberg

MONTEVIDEO. (lat. 34°52′S., long. 56°27′W.)

Early summer (November)

Micropogon furnieri (Desmarest)

Netuma barbus (Lacepède)

where the Signy Island fish were caught, the sea temperature varies between +1·46° and −1·85° C with fluctuations outside this range occurring in shallow water near the shore (personal communication from M. G. White). "Summer fish" were caught from December to March when the sea temperature remained above the approximate freezing point of fish serum (−0·9° C). "Winter fish" were caught from July to October when the sea temperature remained below −1·5° C.

Sea-temperature data are not available for the two South Georgia localities where fish were caught, but the station lists of the Discovery Investigations (1930, 1932, 1941, 1942, 1944*a*, *b*) indicate that the surface-water (0–200 m.) temperatures around South Georgia vary from +4·65° to −1·77° C. South Georgia lies outside the mean northern pack-ice limit although it is south of the Antarctic Convergence, and so South Georgia fish encounter a less rigorous annual temperature regime than Signy Island fish. South Georgia fish were caught in November, during which time South Georgia generally lies between the 0° and 1° C surface isotherms (Mackintosh, 1946). The fish were thus unlikely to be supercooled and were caught under conditions resembling those of a Signy Island summer.

The sea temperature at Montevideo at the time when the South American fish were caught is not known precisely, but was well above the temperature of Antarctic waters.

Capture of fish

Fish were caught on hand lines or long lines, by netting or in baited traps at depths of 1–60 m. in summer and 2–40 m. in winter. Lines were baited with seal, penguin or fish scraps, and traps were baited with whole penguins. At Montevideo and South Georgia, blood samples were taken from fish as soon as they were caught. In summer at Signy Island, fish were kept

until required in aquaria supplied with running sea-water. In winter at Signy Island, fish caught through holes in the sea ice were transported to the laboratory in basins of sea-water and blood samples were taken on arrival. Contact of the fish with ice in the basins was avoided by the addition of enough hot sea-water from vacuum flasks to melt any ice present.

Sampling and storage of serum

Blood from larger fish was obtained by heart puncture using disposable plastic syringes. With smaller fish, particularly *Trematomus newnesi*, the heart was pierced with the fine end of a thin glass tube drawn out to a capillary and blood was extracted by gentle suction. At Montevideo and South Georgia, the blood samples were stored at 0° C until clot shrinkage occurred. Serum was then decanted and deep-frozen for transport to the laboratory on Signy Island. Serum from Signy Island fish was separated from clotted blood by centrifugation.

All samples were stored deep-frozen between analyses. A cloudy white precipitate formed in practically all serum samples when they were deep-frozen for several months. This was possibly due to denaturation of unstable lipoproteins in the serum (Meryman, 1966). The most rapid deterioration occurred in samples which were frequently frozen and thawed. In extreme cases, semi-solid aggregates formed in the samples and an oily smell was noticed. Samples were analysed with minimum delay to avoid possible errors due to this deterioration.

Methods of analysis

Total nitrogen was measured by a semi-micro Kjeldahl method. 0·1 ml. of serum was digested with 3 ml. of concentrated AR sulphuric acid and 100 mg. of Jacob's (1960) catalyst. The resulting ammonia, liberated by excess 40 per cent sodium hydroxide, was steam-distilled into saturated boric acid containing a mixed indicator (Conway, 1962) and titrated with N/100 hydrochloric acid. An ammonium sulphate standard was used.

Protein was determined by the biuret method using the reagent of Gornall and others (1949). 0·1 ml. of serum was mixed with 5 ml. of reagent and after 3·5 hr. (required for maximum colour development) the optical density was measured at 550 nm. against a reagent blank. A blue precipitate (Natelson, 1963) formed in some cases so the solutions were filtered before spectrophotometry. Bovine plasma albumin was used for calibration and so the results are relative rather than absolute, since proteins vary in their response to the biuret reagent. Duplicate analyses agreed to within 0·1 mg./ml. of protein.

Total carbohydrate was determined by the phenol-sulphuric method (Dubois and others, 1956) calibrated with AR glucose. Comparative rather than absolute results were obtained, since it was not possible to allow for non-glucose serum carbohydrate (excessive temperature fluctuations in the laboratory proscribed chromatographic identification of carbohydrates in serum hydrolysates). Duplicate analyses agreed to within 0·1 mg./ml. of glucose.

Reducing sugar was determined by the method of Hagedorn and Jensen (1923*a*, *b*) calibrated with AR glucose. Duplicate analyses agreed to within 0·3 mg./ml. of glucose. Errors due to glycolysis in *Notothenia neglecta* and *N. rossii* serum were estimated by determining, at intervals, reducing sugar concentrations in samples kept at room temperature (10–20° C). The first determinations were made 0·8 hr. after blood was taken from the fish. During the first 10 hr. the reducing sugar decreased by 0·6 mg./ml. (8·5 per cent) in *N. neglecta* serum and by 0·3 mg./ml. (7·1 per cent) in *N. rossii* serum. The results then remained constant until bacterial growth started after 5 days. In a similar experiment, the sera were deep-frozen between determinations and no significant changes in reducing sugar concentration occurred in 16 days. Glycolysis thus occurred only in unfrozen blood or serum. When blood samples were taken, they were allowed to stand at 0° C for 2–3 hr. while clot shrinkage occurred, and so the loss of reducing sugar due to glycolysis in this period could not have exceeded 2–3 per cent before serum samples were separated from the blood clots and deep-frozen.

Sodium and potassium were determined by flame photometry of diluted serum samples. The response of the flame photometer was linear at the low concentrations used so only one standard was necessary for each ion. Samples and standards were alternated when readings were taken and duplicates agreed to within 1 per cent of full-scale deflection, i.e. within 3 mM./l. for sodium and 0·1 mM./l. for potassium.

Chloride was determined by electrometric titration (Ramsay and others, 1955). Samples and standards were measured alternately and duplicates agreed to within 2 mM./l.

Urea was determined by the method of With and others (1961) scaled down for use with 0·1 ml. serum samples. The scaled-down method lacked precision at low urea levels, but the results adequately demonstrated that the urea concentrations in serum are low and hence relatively unimportant in this study.

Comparison of Serum and Pericardial Fluid

Small quantities of colourless pericardial fluid were often obtained from fish when taking blood by heart puncture. These were generally discarded but slight contamination of some of the blood samples undoubtedly occurred. In three cases the volume of pericardial fluid obtained from *Notothenia neglecta* was sufficient for analysis. Pericardial fluid was found to clot on standing and was centrifuged before analysis. Serum samples from the same three fish were also analysed and the results (Table II) showed that pericardial fluid is similar to serum. The slight contamination of serum by pericardial fluid can thus have had little effect on the results of serum analysis. Teleost pericardial fluid does not appear to have been analysed previously so comparison with other species is not possible.

Table II. Comparison of serum and pericardial fluid from *N. neglecta*

	Fish number	*Serum*	*Pericardial fluid*
Freezing point* (°C)	1	−1·14	−1·03
Sodium (mM.)	3	222	232
Potassium (mM.)	3	0·8	0·7
Protein (mg./ml.)	1	59·1	35·0
	2	66·9	51·0
	3	57·8	41·5
Total carbohydrate (mg./ml.)	1	14·1	10·6
	2	13·4	10·4
	3	10·5	7·7
Reducing sugar (mg./ml.)	1	4·3	4·3
	2	5·8	5·5
	3	5·9	5·1

* Determined by method of Ramsay and Brown (1955).

Results of Serum Analysis

The results of serum analysis are shown in Table III. Percentage differences between summer and winter values for *Notothenia neglecta* and *N. rossii* are shown in Table IV. Tables V, VI and VII give comparative results compiled from the literature.

The data show that, in general, Antarctic fish resemble Arctic fish in having more concentrated serum than temperate fish. The high reducing sugar concentration in Signy Island and South Georgia fish is particularly noticeable, The concentrations of serum components in *N. neglecta* and *N. rossii* increased in winter, the total increases being similar in magnitude to those found at low temperatures in other species. When the increases are considered as percentage changes in concentration per °C decrease in temperature, the inorganic serum components are seen to be more affected by temperature in the *Notothenia* species than in other species, whereas serum

TABLE III. CHEMICAL COMPOSITION OF FISH SERUM

(The results are expressed as means, $\bar{x}$. Figures in parentheses are the standard deviation, s, followed by the number of fish in each sample, n. Exceptions are indicated in the footnotes.)

	Na (mM.)	K (mM.)	Cl (mM.)	*Total nitrogen* (mg./ml.)	*Protein* (mg./ml.)	*Total carbohydrate* (mg./ml.)	*Reducing sugar* (mg./ml.)	*Urea* (mg./ml.)
SIGNY ISLAND								
N. neglecta summer	241 (13, 29)	2·1 (1·6, 28)	212 (11, 21)	9·1 (1·3, 30)	61·1 (11·5, 43)	13·0 (2·0, 45)	5·5 (1·4, 34)	ca. 4 (n = 10)
N. neglecta winter	259 (12, 44)	2·9 (1·6, 36)	242 (13, 48)	11·8 (1·3, 12)	71·9 (6·2, 30)	16·3 (2·0, 30)	9·7 (0·9, 30)	ca. 10 (n = 30)
N. rossii summer	212 (17, 14)	2·3 (1·3, 14)	196 (9, 7)	5·4 (0·9, 15)	36·2 (8·6, 19)	8·7 (2·6, 20)	3·1 (0·8, 14)	ca. 7 (n = 9)
*N. rossii** winter	237 (237, 237)	6·2 (5·7, 6·6)	238 (233, 243)	8·8 (8·5, 8·9)	50·0 (48·6, 51·3)	15·2 (14·3, 16·1)	6·0 (5·2, 6·8)	ca. 5 (2, 8)
N. gibberifrons summer	252 (29, 8)	1·9 (1·3, 9)	—	—	52·7 (15·2, 10)	12·5 (2·7, 9)	8·3 (0·7, 3)	—
T. bernacchii† summer	299	0·9	—	—	50·7	8·6	5·2	—
T. newnesi‡ winter	214 (4, 3)	—	227 (7, 3)	—	49·6	18·0	4·8	—
P. charcoti† summer	257	3·1	—	—	70·6	—	—	—
C. aceratus summer	274 (29, 14)	2·1 (1·1, 14)	—	—	59·6 (8·1, 13)	9·5 (3·6, 13)	2·1 (0·6, 13)	—
SOUTH GEORGIA								
N. neglecta early summer	214 (5·7, 5)	0·7 (0·7, 5)	206 (9, 5)	—	55·0 (7·9, 5)	9·2 (1·1, 5)	4·8 (0·2, 5)	— —
N. rossii early summer	209 (10, 30)	1·3 (1·3, 30)	—	—	34·2 (4·4, 30)	8·9 (1·5, 30)	2·4 (0·5, 30)	—
*C. aceratus** early summer	228 (222, 234)	1·7 (1·4, 2·0)	195 (193, 196)	—	27·0 (21·9, 32·0)	3·9 (2·4, 5·4)	0·6 (0·3, 0·9)	—
P. georgianus† early summer	219	1·6	214	—	50·0	9·2	3·3	—
M. microps† early summer	195	4·9	174	—	42·5	7·5	0·8	—
MONTEVIDEO								
*N. barbus**, §early summer	204 (198, 209)	3·5 (2·4, 4·6)	191 (186, 195)	—	41·1 (37·1, 46·0)	6·2 (6·1, 6·2)	0·5 (0·4, 0·5)	—
M. furnieri§, ‖early summer	203	5·6	167	—	31·2	5·3	1·4	—

* Sample consisted of two fish only. The individual results are given in parentheses.
† Sample consisted of one fish only.
‡ Sodium and chloride determinations were carried out on three serum samples which were then combined to provide enough material for the other analyses.
§ Serum freezing points: *N. barbus*, −0·62° C; *M. furnieri*, −0·60° C. Measured by method based on that of Ramsay and Brown (1955).
‖ Serum from three fish combined to provide enough material for analysis.

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TABLE IV. PERCENTAGE CHANGES IN COMPOSITION OF *N. neglecta* AND *N. rossii* SERUM WITH SEASONAL VARIATION IN TEMPERATURE

(The results are given as percentage increases in concentration in winter. Figures in parentheses are percentage increases per °C decrease assuming a seasonal variation of 2·75° C)

	N. neglecta	*N. rossii*
Sodium	7·5 (2·7)	12 (4·4)
Potassium	38 (13·8)	169 (61)
Chloride	14 (5·1)	21·5 (7·8)
Total nitrogen	29·5 (10·7)	63 (22·9)
Protein	17·5 (6·4)	38 (13·8)
Total carbohydrate	25·5 (9·3)	74·5 (27·1)
Reducing sugar	76·5 (27·8)	93·5 (34)

reducing sugar is affected to approximately the same extent in the *Notothenia* species as in the other species.

As well as changes of temperature, changes in the salinity of the sea-water can affect the osmolarity of fish blood (Parry, 1966). At Signy Island, however, the salinity remains practically constant throughout the year with sporadic changes (due to thaws or the formation or break-up of sea ice) occurring relatively quickly and having no long-term effect (personal communication from M. G. White). The possibility that the seasonal changes in *Notothenia neglecta* and *N. rossii* serum were due to salinity changes in the sea-water was therefore rejected and temperature change was implicated as the primary cause. It is relevant to mention at this point that, for the purposes of this study, the fish populations were not subdivided in terms of age, sex, maturity or changes occurring during the reproductive cycle, and the possible effects of these factors on serum composition have not been considered.

The experimental results will now be discussed with particular emphasis on the influence of serum composition on the freezing resistance of the fish.

DISCUSSION

Chemical analysis showed that supercooling of the blood in winter occurred in *Notothenia neglecta*, *N. rossii* and *Trematomus newnesi*. This was confirmed by serum freezing-point and body-temperature measurements (Smith, 1972*a*, *b*). It is reasonable, therefore, to assume that the other species, which were studied only in summer, are supercooled in winter.

If supercooled fish are to survive, their body fluids must not freeze spontaneously and, in addition, the fish must either avoid contact with ice or else be able to resist freezing if contact with ice does occur. These conditions will be considered separately.

Stability of the supercooled state

Supercooled polar fish are undoubtedly stable and spontaneous freezing occurs very infrequently if at all. This is emphasized by the continued survival of such fish.

Dorsey (1948) has shown that supercooled water is surprisingly stable and may be poured, shaken or expelled from a nozzle without freezing, although excessive agitation or friction between suitable interfaces does induce crystallization. Solutes (with the exception of sucrose) were found to increase the stability of supercooled water to some extent by depressing the temperature of frictionally induced freezing to that at which freezing occurred spontaneously due to "motes" in the solution. It is likely, therefore, that the adaptations in blood solute composition peculiar to polar fish are not necessary to stabilize the supercooled state, but are important to protect the fish against "seeding" by contact with ice.

Contact of fish with ice

Signy Island fish were caught at depths of 2–40 m. in winter and it is difficult to estimate the risk of contact of the fish with sea ice or anchor ice. Scuba divers, however, have reported that in winter at Signy Island the only species of fish living in close proximity to and/or in contact with ice is *Trematomus newnesi* which was observed to inhabit clumps of the alga *Desmarestia* on which anchor ice had formed (personal communication from M. G. White). Small shoals of *T. newnesi* have also been seen swimming just under the sea ice near fishing holes. *Notothenia gibberifrons* is restricted in its distribution to depths greater than about 40 m., whereas *N. neglecta* and *N. rossii* are found at depths of 2–40 m. (Everson, 1970). *N. gibberifrons*, therefore, is reasonably certain to avoid ice, whereas *N. neglecta* and *N. rossii* are liable to encounter ice but appear to actively evade contact with it.

Resistance to freezing on contact with ice

Freezing of a supercooled fish's body fluids due to contact with ice may be avoided by three possible methods. These are: (a) the prevention of "seeding" and/or propagation of ice by skin or tissue of unusual thickness or high mechanical strength; (b) the inclusion of an "anti-freeze" in the body fluids to lower their freezing point to that of the environment, and (c) the inclusion in the body fluids of substances at concentrations insufficient to prevent supercooling but sufficient to prevent or retard ice formation if contact with ice occurs.

In the case of Signy Island fish, the first possibility does not occur or else is of limited effectiveness since supercooled fish are killed by contact with ice. The second possibility may also be discounted since supercooling of the fish does occur in winter. The third possibility, however, cannot be discounted and the possible effects of blood solutes on the freezing resistance of fish must be considered. Lusena (1955) studied the crystallization velocity of ice and found that the nucleation and growth rate of ice was markedly hindered in solutions containing substances such as glycerol, proteins and sugars. He also showed that two mechanisms are involved in the retardation of growth rate and that solutes at the concentrations found in biological materials could play a major part in retarding ice-crystal growth in the early stages of freezing. Such an effect may be of major importance in protecting fish against freezing.

The effects of serum composition on the freezing resistance of Signy Island and South Georgia fish can now be considered. The inorganic, organic and macromolecular serum constituents will be discussed separately.

Inorganic serum constituents

Sodium, potassium and chloride concentrations in Antarctic fish sera were measured. Other inorganic serum constituents were ignored, since they are usually present only in low concentrations and are thus of little importance in this study.

Sodium and chloride were found to be the major osmotically active serum constituents and were generally present in only slightly higher concentrations than in the serum of temperate fish. Serum potassium concentrations in the Antarctic fish were small and comparable to those found in temperate fish.

The concentrations of sodium, potassium and chloride in *Notothenia neglecta* and *N. rossii* serum increased slightly in winter but were insufficient to lower the serum freezing point to that of sea-water. Similar changes occur in non-polar fish at temperatures well above freezing. This indicates that such changes are unlikely to be connected specifically with an increased freezing resistance at low temperatures, particularly since inorganic compounds are considerably less effective than organic compounds in retarding ice-crystal growth in solution (Lusena, 1955).

It may be concluded that the inorganic serum constituents contribute little to the freezing resistance of the various species of fish that were studied.

Organic serum constituents

The failure of the inorganic constituents of Antarctic fish blood to provide an obvious defence against freezing focuses attention on the organic (and macromolecular) constituents.

TABLE V. BLOOD COMPOSITION OF SOME MARINE TELEOSTS
(The results are given as means or as ranges of means)

	Sodium (mM./l.)	*Potassium* (mM./l.)	*Chloride* (mM./l.)	*Protein* (mg./ml.)	*Non-protein nitrogen* (mg./ml.)	*Urea* (mg./ml.)	*Carbo-hydrate* (mg./ml.)	*Footnotes*
Gadus callarias	180	6·5	164	—	—	—	—	1,2,3
Pollachius virens	180	4·6	158	—	—	—	—	1,3
Labrus bergylta ♂	195	8·1	100	—	—	—	—	1,3
Labrus bergylta ♀	157	10·8	78	—	—	—	—	1,3
Lophius piscatorius ♂	204	8·9	173	—	—	—	—	1,3
Lophius piscatorius ♀	193	7·0	200	—	—	—	—	1,3
Marine teleosts	—	—	—	—	—	0·3	—	1,3
Brevoortia tyrannus	—	—	147	—	0·73	0·017	0·9	1,3,4
Myoxocephalus aenems	—	—	—	—	0·56	0·019	—	3,4
Tantoga anitis	—	—	—	—	0·60	0·018	—	3,4
Tantogolabrus	—	—	—	—	0·63	0·021	—	3,4
Muraena helena	211·8	1·95	188·4	80	—	—	—	5,6
Scomberomerus maculatus	188	9·8	167	35	—	—	—	7,8
Thunnus thynnus	190	26·8	181	65	—	—	—	7,8
Mycteroperca venenosa	190	6·4	181	23	—	—	—	7,8
Sphyraena barracuda	215	6·4	189	33	—	—	—	7,8
Mycteroperca bonaci	228	7·9	208	28	—	—	—	7,8
Promicrops itaiara	200	5·8	166	56	—	—	—	7,8
Salmo trutta	166	3·5	138	—	—	—	—	9,10
Oncorhynchus nerka ♂	—	—	—	—	0·367	—	2·065	11,12
Oncorhynchus nerka ♀	—	—	—	—	0·350	—	2·093	11,12
Opsanus tau	—	—	—	—	—	—	0·2–1·0	3,13
Fundulus heteroclitus	183·0	4·76	145·8	43·5–43·8	0·595–0·666	0·24	0·696 (0·249) U 1·624 (0·585) F	8,10,14

[1] Vinogradov, 1953. [2] *Gadus callarias* = *Gadus morhua*. [3] Values for blood. [4] Denis, 1922. [5] Robertson, 1954. [6] Concentrations (except protein) in mg. ions/kg. plasma water. [7] Becker and others, 1958. [8] Values for serum. [9] Gordon, 1959. [10] Sea-water adapted fish. [11] Jonas and MacLeod, 1960. [12] Values for plasma. [13] Nace and others, 1964. [14] Pickford and others, 1969. Carbohydrate values in mg./ml. glucose with mg./ml. non-glucose carbohydrate in parentheses. U indicates unfed fish and F indicates fish fed 2 hr. before autopsy.

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TABLE VII. PERCENTAGE CHANGES IN COMPOSITION OF MARINE TELEOST BLOOD WITH CHANGES OF ENVIRONMENTAL TEMPERATURE

(The results are given as percentage increases in concentration over the indicated temperature range. Figures in parentheses are percentage increases in concentration per °C decrease in temperature.)

	Temperature change (°C)	Na	K	Cl	*Protein*	*Non-protein nitrogen*	*Glucose*	*Non-glucose carbohydrate*	*Footnotes*
G. ogac, *M. scorpius*, *S. alpinus*	6·73 (summer to winter)	—	—	33 (4·9)	—	—	—	—	1
G. ogac, *M. scorpius*	6·73 (shallow to deep water)	—	—	22·5 (3·3)	—	—	—	—	1
G. ogac	0 (presence of ice)	—	—	36	—	—	—	—	1
M. tomcod	5·5 (9½ days at −1·5°)	6·5 (1·2)	63 (11·5)	17 (3·1)	—	33·5 (6·1)	—	—	2
G. morhua	Above 2° to below 2° Nov./Dec. 1954	14	62	5·5	—	—	—	—	3,4
	Above 2° to below 2° June 1955	10·5	16·5	4	—	—	—	—	3,4
	Above 2° to below 2° July 1955	−1	−11	0	—	—	—	—	3,4
G. callarias	10·5 (15° to 4·5°)	—	—	10 (1·0)	—	—	—	—	4,5,6
	6 (4·5° to −1·5°)	—	—	5·5 (1·0)	—	—	—	—	4,5,6
	16·5 (15° to −1·5°)	—	—	16 (1·0)	—	—	—	—	4,5,6
	~10 (2–14 days at 0° to −1·4°)	—	—	8 (0·8)	—	—	—	—	4,6
	~10 (60 days at −1·4°)	—	—	14·5 (1·5)	—	—	—	—	4,6
C. lumpus	5·5 (10° to 4·5°)	—	—	12 (2·2)	—	—	—	—	4,5,6
	6 (4·5° to −1·5°)	—	—	18 (3)	—	—	—	—	4,5,6
	11·5 (10° to −1·5°)	—	—	32 (2·8)	—	—	—	—	4,5,6
P. americanus	Summer to winter	30	—	27·5	—	—	—	—	7
O. tau	~13 (summer to winter)	—	—	—	—	—	480 (37)	—	8
F. heteroclitus	10 (20° to 10°)	0	0	0	0	0	0	—	9
	8 (10° to 2°)	7·4 (0·9)	0	15·5 (1·9)	0	41·3 (5·2)	119·1 (14·9)	—	9
	3 (2° to −1°)	0	0	0	0	0	146·5 (48·8)	—	9
	21 (20° to −1°)	7·4 (0·4)	0	15·5 (0·7)	0	41·3 (2·0)	440·4 (21)	—	9
	21·5 (20° to −1·5°)	12 (0·6)	0	17 (0·8)	—	—	—	—	10
	6 (10° to 4°)	—	—	—	—	—	37·2 (6·2)	—	11
	5·5 (4° to −1·5° for 1 day)	—	—	—	—	—	203 (37)	—	11
	5·5 (4° to −1·5° for 10 days)	8 (1·5)	—	—	—	—	157·2 (28·6)	—	10,11
	5·5 (4° to −1·5° for 15 days)	—	—	—	—	—	338 (61·5)	—	11
	5·5 (4° to −1·5° for 25 days)	—	—	—	—	—	99 (18·0)	—	11
	5·5 (4° to −1·5° for 50 days)	10·4 (1·9)	—	—	—	—	—	—	10
	21·5 (20° to −1·5°)	—	—	—	—	—	—	0	11
	21·5 summer fish	—	—	—	—	—	247 (12·7)	—	12
	21·5 winter fish	—	—	—	—	—	513 (23·9)	—	12

	Mg	Ca	HCO_3	*Cholesterol*	*Inorganic phosphate*	
10 (20° to 10°)	0	0	0	0	0	9
8 (10° to 2°)	38·5(4·6)	43·8(5·5)	0	73·1(9·1)	0	9
3 (2° to −1°)	0	0	51·1(17·0)	0	0	9
21 (20° to −1°)	38·5(1·8)	43·8(2·1)	51·1(2·4)	73·1(3·5)	0	9
21·5 (20° to −1·5°)	33 (1·5)	30 (1·4)	11 (0·5)	—	0	10

[1] Scholander and others, 1957. [2] Gordon and others, 1962. [3] Woodhead and Woodhead, 1959, 1965. [4] *G. morhua* = *G. callarias*. [5] Eliassen and others, 1960 [6] Leivestad, 1965. [7] Pearcy, 1961. [8] Nace and others, 1964. [9] Umminger, 1967. [10] Umminger, 1969*a*. [11] Umminger, 1968. [12] Umminger, 1969*b*.

TABLE VI. VARIATION IN COMPOSITION OF MARINE TELEOST BLOOD WITH CHANGES OF ENVIRONMENT

(The results are given as means. In cases where data were presented in diagrammatic form only, the means were calculated from measurements of individual points on the graphs. Discrepancies between tabulated values and corresponding points on the graphs for some results of Eliassen and others (1960) and Leivestad (1965) indicate possible errors in means calculated from the two graphs for which the relevant data are not tabulated.)

		Na (mM./l.)	K (mM./l.)	Cl (mM./l.)	*Protein* (mg./ml.)	*Non-protein* N (mg./ml.)	*Urea* (mg./ml.)	*Carbo-hydrate* (mg./ml.)	*Footnotes*
Shallow-water fish	*Gadus ogac*, *Myoxocephalus scorpius*, *Salvelinus alpinus*: Summer, +5°	—	—	185	—	—	—	—	1,2
	Gadus ogac, *Myoxocephalus scorpius*, *Salvelinus alpinus*: Winter, −1·73°	—	—	246	—	0·9	—	—	1,2
	G. ogac, *M. scorpius*: Summer, −1·73°, 4–8 days	—	—	227	—	—	—	—	1,2
	G. ogac: Winter, −1·73° in presence of ice	—	[illegible]	335[illegible]	—	—	—	—	1,2
Deep-water fish	*Boreogadus saida*, *Lycodes turneri*, *Liparis koefoedi*, *Gymnocanthus tricuspis*, *Icelus spatula*: All year, −1·73°	—	—	223	—	—	—	—	1,2
M. scorpius	New Brunswick, spring, +4°	276	6·4	184	—	1·7	—	—	3,4
	Labrador, spring, −1·7°	216	4·3	234	—	1·3	—	—	3,4
G. ogac	Labrador, spring, −1·7°	216	5·5	243	—	4·0	—	—	3,4
Microgadus tomcod	New Brunswick, spring, +4°	231	5·1	142	—	1·0	—	—	3,4
	New Brunswick, spring, −1·5° (9½ days)	246	8·3	166	—	1·3	—	—	3,4
G. morhua (= *G. callarias*)	Cod distribution limited at 2°, Above 2°, Nov./Dec. 1954	184	4·5	178	—	—	—	—	2,5
	Cod distribution limited at 2°, Above 2°, June 1955	191	4·2	178	—	—	—	—	2,5
	Cod distribution limited at 2°, Below 2°, Nov./Dec. 1954	210	7·3	188	—	—	—	—	2,5
	Cod distribution limited at 2°, Below 2°, June 1955	211	[illegible]	18[illegible]	—	—	—	—	2,5
	Cod distribution not limited at 2°, Above 2°, July 1955	197	4·5	174	—	—	—	—	2,5
	Cod distribution not limited at 2°, Below 2°, July 1955	195	4·0	174	—	—	—	—	2,5
G. callarias	Sea temperature +15°	—	—	162	—	—	—	—	2,6,7
	Sea temperature +4·5°	—	—	178	—	—	—	—	2,6,7
	Sea temperature −1·5°	—	—	188	—	—	—	—	2,6,7
Cyclopterus lumpus	Sea temperature +10°	—	—	165	—	—	—	—	2,6,7
	Sea temperature +4·5°	—	—	185	—	—	—	—	2,6,7
	Sea temperature −1·5°	—	—	218	—	—	—	—	2,6,7
Anarhichas minor	Sea temperature −1·5°	—	—	195	—	—	—	—	2,6,7
Drepanopsetta platessoides	Sea temperature −1·5°	—	—	210	—	—	—	—	2,6,7
G. callarias	Laboratory acclimation 8–10°	—	—	163	—	—	—	—	2,7
	Laboratory acclimation 0° to −1·4° (2–14 days)	—	—	176	—	—	—	—	2,7
	Laboratory acclimation −1·4° (60 days)	—	—	187	—	—	—	—	2,7
Pseudopleuronectes americanus	Summer	161	—	152	—	—	—	—	2,8
	Winter	209	—	194	—	—	—	—	2,8
Opsanus tau	Summer, between 11° and 22°	—	—	—	—	—	—	0·2	9
	Winter, between 6° and −1°	—	—	—	—	—	—	1·16	9
Trematomus bernacchii	Shallow water (20 m.)	—	—	254	19·0	4·81	1·1	8·80	4,10,11,12
	Deep water (300 m.)	—	—	254	11·2	3·43	—	5·87	4,10,11,12
Trematomus bernacchii	Oct./Nov. Between +3·8° and −2·0° in laboratory	—	—	274	—	—	—	—	2,13
	Dec. 0° to −1·9° for varying times in laboratory	—	—	254	—	—	—	—	2,13
T. hansoni	Shallow water (20 m.)	—	[illegible]	259	18·3	4·80	—	8·38	4,10,11,12
	Deep water (300 m.)	—	[illegible]	25[illegible]	16·2	3·75	—	5·94	4,10,11,12
T. borchgrevinki	Shallow water (20 m.)	—	—	235	18·9	5·04	1·3	8·31	4,10,11,12

[1] Scholander and others, 1957. [2] Values for plasma. [3] Gordon and others, 1962. [4] Values for serum. [5] Woodhead and Woodhead, 1959, 1965. Correction factor added to chloride values (Woodhead and Woodhead, 1965, p. 722). [6] Eliassen and others, 1960. [7] Leivestad, 1965. [8] Pearcy, 1961. [9] Nace and others, 1964. Values for blood. [10] DeVries and Wohlschlag, 1969; DeVries, 1970. [11] Mean freezing point of shallow water was −1·90° C. Average sea temperature throughout the year was −1·87° C. [12] Filtrate of 5 per cent serum in 10 per cent trichloroacetic acid used for all analyses except chloride. [13] Potts and Morris, 1968.

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The Antarctic species studied in winter, unlike certain inshore Arctic fish (Gordon and others, 1962), did not contain a specific organic "antifreeze" in their serum, and so organic compounds which contribute to the freezing resistance of the fish must do so by preventing or retarding nucleation and propagation of ice in the fish.

The most outstanding feature of Antarctic fish serum was the unusually high concentration of reducing sugar found in nearly all species studied. Similar high concentrations have been found in supercooled *Fundulus heteroclitus* (Umminger, 1967, 1968, 1969*a*, *b*, *d*) and many reports in the literature (Meryman, 1966; Umminger, 1969*d*) relate high concentrations of carbohydrate or other hydroxyl-containing compounds to the ability to supercool and/or survive freezing injury. In *Notothenia neglecta* and *N. rossii* serum, the high summer reducing sugar concentrations practically double in winter despite the relatively small temperature difference between summer and winter (approximately 2·75° C).

The inference is that the high reducing sugar concentrations are related to the freezing resistance of the Antarctic fish and may be effective in preventing their blood from freezing if the supercooled fish come into contact with ice.

Macromolecular serum constituents

Low concentrations of proteins retard the crystallization velocity of ice in supercooled solutions as effectively as alcohols and sugars (Lusena, 1955). By this means the proteins in Antarctic fish serum (which are present in concentrations similar to those in temperate fish serum) may contribute towards the freezing resistance of the fish. A glycoprotein isolated from various *Trematomus* species at McMurdo Sound is of particular interest in this respect (DeVries and Wohlschlag, 1969; DeVries, 1970). The glycoprotein, despite its high molecular weight, appeared to act as a freezing-point depressant and was particularly effective at low concentrations. Theories of the colligative properties of solutions cannot account for this. It is, however, possible that the anomalous freezing-point depressions (measured with a Fiske osmometer) were due to retardation of the crystallization velocity of ice by the glycoprotein. This is considered in detail in a separate paper (Smith, 1972*a*). The glycoprotein, however, whether or not it is a true freezing-point depressant, undoubtedly helps to protect the McMurdo Sound *Trematomus* species against freezing.

An indication of the relative glycoprotein content of serum is given by the difference between the reducing sugar and total carbohydrate concentrations, i.e. a comparative measure of non-reducing chemically bound carbohydrate. Table VIII shows the results of this procedure applied to the Signy Island, South Georgia and Montevideo fish. Of the Antarctic species, *Trematomus newnesi* has a high concentration of bound serum carbohydrate, whereas the other Antarctic species have amounts varying from slightly less to nearly twice as much as the Montevideo fish. It is reasonable to assume that the marked freezing resistance of *T. newnesi* is related to its high serum glycoprotein concentration.

It may be tentatively concluded that serum proteins in general contribute to the freezing resistance of the Antarctic fish, and that serum glycoproteins in particular, depending on their concentrations, may be of particular importance in this respect.

Relation of serum composition to freezing resistance—comparison of species

The freezing resistance of the various Antarctic fish varies considerably (Smith, 1972*b*). *Trematomus newnesi* is perhaps the most resistant and is the only one of the species studied that does not avoid contact with ice. *Notothenia neglecta* is moderately resistant and can survive contact with ice for several hours when supercooled. *N. rossii* and *N. gibberifrons* have a negligible resistance to freezing and are quickly killed by contact with ice when supercooled. Specimens of the three *Notothenia* species that died of freezing were examined and in no case was there evidence of ice formation in the blood or heart.

The high resistance to freezing of *Trematomus newnesi* may be related to the unusually high concentration of serum glycoprotein in this species, since the other serum components were present in concentrations comparable to those in the other Antarctic fish species. There were no obvious differences in the serum composition of *Notothenia neglecta* compared with *N. rossii* and *N. gibberifrons* that can account for the moderate freezing resistance of *N. neglecta*.

TABLE VIII. CHEMICALLY BOUND CARBOHYDRATE IN FISH SERUM

(Results expressed as difference between total carbohydrate (mg./ml.) and reducing sugar (mg./ml.) assuming that all bound carbohydrate is non-reducing.)

	Bound carbohydrate (mg./ml.)
SIGNY ISLAND	
N. neglecta (summer)	7·5
N. neglecta (winter)	6·6
N. rossii (summer)	5·6
N. rossii (winter)	9·2
N. gibberifrons (summer)	4·6
T. newnesi (winter)	13·2
T. bernacchii (summer)	3·4
C. aceratus (summer)	7·4
SOUTH GEORGIA (November)	
N. neglecta	4·4
N. rossii	6·5
C. aceratus	3·3
P. georgianus	5·9
M. microps	6·7
MONTEVIDEO (November)	
N. barbus	5·7
M. furnieri	3·9

This is not unexpected, however, since in all three *Notothenia* species the blood is equally resistant to freezing when supercooled fish are killed by contact with ice. Reducing sugar is probably of major importance in preventing ice formation in supercooled blood with an additional contribution from the serum protein and glycoprotein. It must be emphasized, however, that a causal relationship between serum composition and freezing resistance is merely implied and not proven.

Notothenia neglecta, *N. rossii* and *N. gibberifrons*, whose blood is resistant to freezing, may be contrasted with deep-water McMurdo Sound fish. Mortality of the McMurdo Sound fish was found to be high when they were held in live boxes just below the sea ice and ice formed in the blood (and urine) of the fish (Wohlschlag, 1964). Obviously, the blood of these fish was not well protected against freezing.

The inability of the three *Notothenia* species to survive contact with ice when supercooled shows that the high freezing resistance of the blood does not necessarily extend to the rest of the body. Thus, while it may be predicted that the blood of the other Antarctic species studied is likely to be resistant to freezing, it is not possible to predict, on the basis of serum composition alone, whether a supercooled fish would survive contact with ice.

CONCLUSIONS

The conclusions of this study may be stated briefly as follows:

i. The serum of the Antarctic fish species that were studied was found in general to be a slightly more concentrated solution than the serum of temperate fish. Unusually high concentrations of serum reducing sugar were found in most Antarctic species.

ii. Concentrations of most serum constituents increased in winter in the two species that were studied both in summer and winter. The increases were insufficient to lower the serum freezing point to that of sea-water.

iii. Inorganic serum constituents were considered unlikely to contribute significantly to the freezing resistance of the fish. Organic and macromolecular serum constituents, particularly reducing sugar and possibly proteins and glycoproteins, were considered likely to protect the blood of supercooled fish from freezing on contact of the fish with ice by preventing or retarding the nucleation and propagation of ice crystals in the blood.

iv. Comparison of serum composition and freezing resistance of several species showed that a knowledge of serum composition alone was insufficient to predict the survival time of a supercooled fish in contact with ice, but was possibly sufficient to predict whether or not the blood of the fish was liable to freeze in these circumstances.

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