adopted in order to use animals old enough to be immunologically responsive but young enough to be highly susceptible.

Antigen was prepared by saponization of heavily infected heparinized rat blood according to the method of Zuckerman *et al.* (*3*). The liberated, washed parasites were crushed in a Hughes-Colab press, and the cell-free extract was obtained by centrifugation at 15,000*g*. Protein content as determined by Biuret analysis ranged from 1.3 to 2.1 g per 100 ml of extract.

Adult female rats (approximately 250 g) infected with *P. berghei* 2 to 4 months earlier were reinoculated with rat blood containing 1×10^6 parasitized erythrocytes and mated 1 week later, to produce offspring of immune mothers. Litters of immune and normal mothers were weaned when they were 50 g and divided into two groups each. One group received 0.2 ml of antigen subcutaneously. The other group remained as unvaccinated controls. All rats were challenged 1 week later, when they weighed approximately 75 g, with 1×10^6 parasitized erythrocytes inoculated intraperitoneally. A thin blood film from each animal was made daily and stained with Giemsa; 200 erythrocytes were examined to calculate the percentage of parasitized to normal erythrocytes. The experiment was repeated four times.

The results of the four experiments are summarized in Table 1. Maternal immunity alone conferred protection in that the mortality rate was significantly lower ($P < .01$) than in the control group from normal mothers. However, the parasitemia was relatively high in many rats born of immune mothers, as reflected by the average peak parasitemia of 32 percent. Maternally derived antibody also tended to delay the onset of patency. The single injection of antigen had relatively little effect on the peak parasitemia or prepatent period in progeny of normal mothers. However, the mortality rates were lower ($P < .02$) than in unimmunized control rats, and presumably the immune response was sufficient to enable a large proportion to survive the infection. The vaccinated progeny of immune mothers exhibited a higher level of protective immunity than their unvaccinated littermates. The mortality rate was significantly lower ($P < .01$) in the vaccinated group, no deaths occurring in any of the vaccinated rats. Moreover, the average peak parasitemia was only one-fourth that of the unvaccinated group born of immune mothers. The prepatent period was prolonged another day, and the peak parasitemia was transient, lasting usually not more than 24 or 48 hours. After crisis, only the occasional parasite could be found in the daily blood films.

In areas of holoendemic malaria, the segment of the population most at risk to the serious consequences of the infection is the young children and infant-toddler group. This special age-related susceptibility has generally been attributed to the relatively ineffective immunologic response of young children to malaria. The results of these experiments indicate that young experimental animals may acquire a high level of protective immunity through the combined effect of maternal immunity and vaccination. Whether this phenomenon holds true for young children is not as yet known, nor have the optimal factors of maternal immunity and immunization that promote the maximal protective immunity been elucidated.

Production of a sterile immunity via the combination of maternal immunity and immunization is probably not feasible nor necessarily desirable. It has been argued (*4*) that the most beneficial effect of protective immunity would be to suppress the primary parasitemia so that it is of low-grade transient character without attendant clinical manifestations. A state of premunition would then ensue with a very scanty parasitemia to maintain long-term functional immunity. This has been the pattern of events noted in these experiments, brought about by the combined agencies of maternally derived immunity and immunization with a nonliving vaccine.

ROBERT S. DESOWITZ
Section of Tropical Medicine and Medical Microbiology, School of Medicine, University of Hawaii, Honolulu 96816

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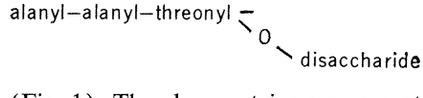
Glycoproteins as Biological Antifreeze Agents in Antarctic Fishes

Abstract. *The blood serums of Antarctic fishes freeze at −2°C, which is approximately 1°C below the melting points of their serums. This thermal hysteresis is due to the influence of serum glycoproteins. The temperatures of freezing and melting of aqueous solutions of the purified glycoproteins suggest that this thermal hysteresis results from the adsorption of the glycoprotein molecule onto the surface of ice crystals.*

Certain Antarctic notothenid fishes, notably *Trematomus borchgrevinki, T. bernacchii,* and *T. hansoni,* are able to avoid freezing, even though they spend much of their lives in water laden with minute ice crystals (−1.9°C) and may, in fact, rest upon masses of anchor ice (*1–4*). The blood serums of these fishes freeze at approximately −2°C, a temperature at which the fishes die from freezing (*2, 3, 5*). The unusually low temperatures at which the blood serums freeze have been shown to result partly from the presence of a group of glycoproteins (*1, 2, 6*). On a weight basis, these glycoproteins lower the freezing point of the solution as effectively as NaCl, although the glycoprotein molecules are several hundred times larger than NaCl (*1, 6*). These glycoproteins, which occur in the serums of most Antarctic fishes, have been isolated and characterized (*1, 2, 6–8*). They are composed only of alanine, threonine, *N*-acetylgalactosamine, and galactose. These subunits are organized into repeating units with the sequence:



(Fig. 1). The glycoproteins are present in several sizes (molecular weights = 10,500, 17,000, and 21,500), and data from physical studies indicate that they are expanded structures without α-helical or β-structure (*6*). In attempts to elucidate the mechanism by which these glycoproteins so effectively lower the temperature at which ice formation occurs, both physical and chemical approaches have been employed.

In this study the method of Ramsay and Brown (*9*) was used to determine freezing points of blood serums collected from the Antarctic notothenid fishes *Trematomus borchgrevinki, Notothenia coriiceps*, and *N. gibberifrons*, as well as the chaenichthyid fish *Chaenocephalus aceratus*, and aqueous solutions of the glycoproteins which were isolated from the blood serum of *T. borchgrevinki*. Samples were placed in capillary tubes [40 by 0.75 mm (outside diameter)] which were closed at one end and plugged with heavy mineral oil at the other end. The capillary tubes were then frozen with Dry Ice and thawed in a refrigerated bath until only one ice crystal approximately 0.25 mm in diameter remained and neither melted nor increased in size. The temperature of the bath was then raised or lowered at the rate of 0.01°C per 5 minutes, and the temperature at which the ice crystal began to slowly decrease or increase in size was taken as the melting or freezing point, respectively. According to this method, the freezing points of (or temperatures at which ice formation occurred in) aqueous solutions of the glycoproteins were shown to be different from the melting points (Fig. 2). The difference varies with the concentration of the glycoprotein in solution; that is, at 4 mg per milliliter the difference is 0.4°C, whereas at 12 mg per milliliter the difference is 0.8°C. The difference between the melting and freezing points in solutions of the glycoproteins contrasts sharply with the thermal behavior of salt solutions, for which the melting and the freezing points are the same (*10*).

Previous studies, in which enzymatic degradations and chemical modifications of the glycoproteins were carried out, indicate that this unique "antifreeze" property has an absolute dependence upon molecular integrity. For example, when the proteolytic enzyme subtilisin (subtilopeptidase A) cleaves as few as three bonds in the polypeptide backbone, complete inactivation results (*6*). Acetylation of as few as 30 percent of the hydroxyl groups on the carbohydrate moiety also results in complete inactivation, as is the case if the galactose residues of the carbohydrate moiety are subjected to periodate oxidation (*8*).

Since the modification experiments all implicate the hydroxyl groups in an active role, I studied the effect of borate, which complexes readily with the hydroxyl groups of most sugar residues, on the "antifreeze" activity of the glycoproteins. In the presence of 0.15*M* sodium borate, the glycoproteins possess no "antifreeze" activity (Fig. 3). If the borate is removed from the glycoprotein-borate solution by dialysis against a moving stream of distilled water, the "antifreeze" activity of the glycoproteins is completely restored. Borate is known (*11*) to complex most readily with hydroxyl groups that are on adjacent carbons and, in addition, are in a *cis*, rather than a *trans*, configuration. In the case of the glycoproteins the borate is presumably complexing with the *cis*-hydroxyl groups of the galactose residues. More concrete evidence that the *cis*-hydroxyl groups are the "active sites" comes from freezing experiments on solutions of glycoproteins in which the alcohol group on carbon 6 of the galactose residues has been oxidized to an aldehyde by the enzyme D-galactose oxidase (Worthington). On the basis of a galactose standard, this enzyme converts about 90 percent of the galactose residues to the oxidized form. Such treatment has no effect on the "antifreeze" activity, an indication that the hydroxyl group of carbon 6 is not essential for activity. When the oxidized glycoproteins are treated with borate, the "antifreeze" activity is again lost but can be restored upon dialysis as before. Thus, on the basis of experiments in which periodate oxidation is carried out, which eliminates carbon 3 of the galactose residue as formic acid but does not react with the *N*-acetylgalactosamine (*8*), one can exclude the hydroxyl groups of the *N*-acetylgalactosamine residues from an active role and thus identify the hydroxyl groups of carbons 2, 3, and 4 of the galactose residues as "active sites." The data from the borate experiments permit one to decide, with reasonable confidence, that the active role is associated with the *cis*-hydroxyl groups of carbons 3 and 4 (Fig. 1).

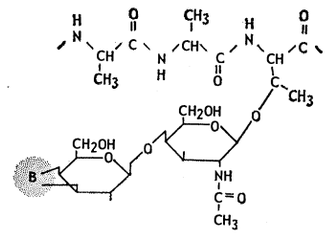


Fig. 1. Structure of glycoproteins with "antifreeze" properties. The glycoproteins, of various molecular weights (10,500, 17,000, and 21,500), are made up of repeating units of this structure. In the presence of 0.15*M* sodium borate the glycoproteins demonstrate no "antifreeze" activity, presumably because the borate (B) is complexed with the *cis*-hydroxyl groups of carbons 3 and 4 of the galactose residues. Removal of the borate by dialysis restores the "antifreeze" activity.

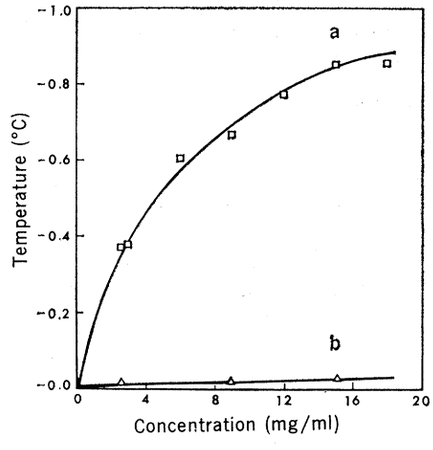


Fig. 2. Comparison of the freezing and melting points of aqueous solutions of the glycoproteins isolated from the serums of *Trematomus borchgrevinki* as a function of concentration. Curve a represents the temperatures of ice crystal growth in the presence of a seed crystal (freezing point); curve b shows the melting points of the last ice crystals. The temperature of the bath was lowered or raised at the rate of 0.01°C per 5 minutes.

The presence of a thermal hysteresis in the freezing behavior was also observed with the blood serums of the Antarctic fishes (Table 1). The temperature at which a small ice crystal in a capillary tube of serum began to increase in size upon slow cooling was found not only to vary from one determination to another, as did the melting point, but also to be 1.0° to 1.7°C lower than the serum melting point in the case of *T. borchgrevinki*. In Table 1 the highest temperatures of ice crystal growth in the blood serums are nearly the same as the organismal freezing temperatures. These are also very close to the temperatures obtained when the Fiske osmometer is used to determine the freezing points of the blood serums from these fishes (*1–3*).

The occurrence of a thermal hysteresis in the freezing behavior of both the serums and aqueous solutions of the glycoproteins suggests the lack of a rapid approach to equilibrium, and thus prevention of the measurement of the true equilibrium thermodynamic parameter (the freezing point). Since solutions of the glycoproteins can be lowered to temperatures as much as 0.8°C below the melting points of the ice crystals present before freezing occurs, a kinetic interpretation, that is, a de-

Table 1. Comparison of the lowest temperatures of survival of several Antarctic fishes in the presence of ice crystals with the freezing behavior of pooled samples of their blood serums. Numbers of specimens are shown in parentheses.

Species	Organismal freezing temperature* (°C)	Melting point (°C) [method of Ramsay and Brown (*9*)]	Freezing point (°C)	
			Lowest temperature of ice crystal growth†	Highest temperature of ice crystal growth†
Trematomus borchgrevinki	−2.1° (5)	−1.05° to −1.10°	−2.76°	−2.08°
Notothenia gibberifrons	−1.9° (6)	−1.10° to −1.07°	−2.52°	−2.04°
Notothenia coriiceps	−1.8° (5)	−0.95° to −1.05°	−2.21°	−1.92°
Notothenia coriiceps	−1.7° (1)			−1.86°
Chaenocephalus aceratus	−1.2° (5)	−0.90° to −1.05°	−1.60°	−1.30°

* The freezing temperatures of the whole organisms were obtained by slowly lowering the temperature (0.1°C per hour) of a refrigerated 60-liter aquarium in which finely crushed ice was suspended and noting the temperatures at which the specimens froze. Death was preceded by convulsions, and the temperature at which the fish lay motionless on its back with opercular flared was taken as the temperature of freezing. † The temperature of ice crystal growth was determined in the presence of a small seed crystal about 0.1 mm in diameter.

creased rate of ice crystal growth, might seem to be in order. However, when a solution of the glycoproteins (12 mg per milliliter) that has a freezing point of −0.8°C is incubated in a capillary tube for 24 hours at −0.75°C in the presence of an ice crystal 0.1 mm in diameter, there is a lack rather than a slow rate of crystal growth. This same thermal stability was observed with the serums of all the Antarctic fishes studied; for example, after 24 hours at −2.0°C in the presence of a seed crystal, no ice propagation was observed in blood serum from *T. borchgrevinki*. A kinetic interpretation also presents a biological problem: even in *T. borchgrevinki* serum, the melting point (−1.1°C) is well above the warmest summertime water temperature (−1.5°C) ever recorded in McMurdo Sound (*12*), and thus a means of melting small ice crystals as they form would be necessary. Deep body temperatures of several notothenid fishes were found to be only 0.02° to 0.1°C higher than that of the surrounding water (*2, 3, 13*); these data do not suggest the existence of a "thermal site" for melting ice if it does form slowly.

Thus the question of how the glycoproteins achieve their "antifreeze" effect remains. At one time it was suggested that they prevent freezing by structuring water, a hypothesis that seemed consistent with the large number of hydroxyl groups present on the carbohydrate moieties (*6*). However, data from freezing experiments with solutions of the borate-complexed glycoproteins indicate that only the *cis*-hydroxyl groups of the galactose residues are associated with "antifreeze" activity. The limited number of "active sites" leads one to suggest that the glycoproteins do not prevent the growth of ice crystals by structuring water. A water-structuring hypothesis should not be completely excluded, however, because many solutes participate in the formation of crystalline clathrate hydrates. In the case of methane, the water lattice contains 6.7 molecules of water per molecule of methane (*14*). However, the guest molecules of clathrate structures tend to be small, to be apolar, and to support ice-like crystalline structures at temperatures above 0°C, properties that are in contrast to those of the glycoproteins which lower only the temperature at which ice forms. An alternate and perhaps more attractive hypothesis is that the glycoproteins exert their "antifreeze" effect by being adsorbed onto the surface of ice crystals, thereby preventing water molecules from settling into the ice lattice of the crystal until a much lower temperature is reached. The fact that the ice crystals melt at their "normal" (or expected) melting temperature suggests that, if the glycoproteins are adsorbed onto the surface of the ice crystals, these molecules do not impede the movement of water molecules from the ice crystal into solution.

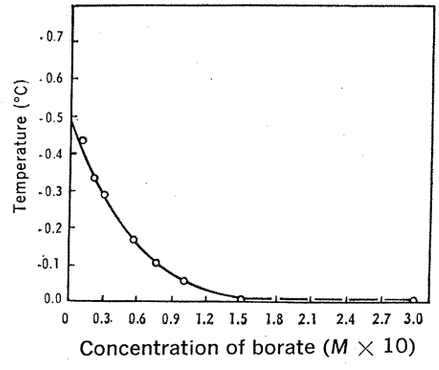


Fig. 3. Freezing points of aqueous solutions of the glycoproteins (4.5 mg per milliliter) in the presence of sodium borate, showing the amount of borate which reversibly inactivates the glycoproteins with "antifreeze" activity. The contribution of the sodium borate to the lowering of the temperature of freezing has been subtracted for each of the freezing-point determinations, which were made with a Fiske freezing-point osmometer. At low concentrations (4.5 mg per milliliter) the freezing points determined with the Fiske osmometer are nearly the same as those determined by the method of Ramsay and Brown (*9*).

If the role of the glycoproteins is that of a surface deactivator preventing crystal growth, then the melting point data of the blood serums must be reconciled with such a hypothesis, because any ice formation in the body fluids would lead to death. All available data indicate that no ice formation occurs in the body, a result apparently attributable to the activity of the glycoproteins. It seems that the glycoproteins are somehow presenting a barrier to ice crystal propagation across the cell membrane. Even when *T. borchgrevinki* is held at temperatures near −2.0°C, a temperature at which ice crystals are continually passed over the gill filaments, the fish does not freeze.

Although the abnormal freezing behavior of solutions of the glycoproteins (Fig. 2) is quite unusual, similar freezing phenomena have been reported for other animals. A hysteresis of as much as 2° to 10°C in the freezing temperature has been reported by Ramsay (*15*) for the fluid taken from the anterior perinephric space in the mealworm *Tenebrio molitor*. The component responsible for the abnormal freezing behavior in this organism is proteinaceous (activity destroyed by trypsin) but has not been characterized other than by its molecular weight, which ranges from 10,000 to 12,000 (*16*).

The melting and freezing points of the plasma of the Arctic fishes *Myoxocephalus scorpius* and *Gadus ogac* have been observed to differ by as much as 0.1°C (*17*). Although the solutes responsible for this behavior have not been identified, it is possible that the

serums of these fishes may have glycoproteins similar to those present in the blood of the Antarctic fishes.

ARTHUR L. DEVRIES
Physiological Research Laboratory, Scripps Institution of Oceanography, University of California, San Diego, La Jolla 92037

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Coral-Eating Sea Stars Acanthaster planci in Hawaii

Abstract. *An aggregation of 2 × 10⁴* Acanthaster planci *was observed from September 1969 to November 1970. The sea stars within the aggregation were very uniform in size, and their reproduction was seasonal. Their average diameter and weight also varied seasonally in a manner that suggests a correlation between average size and breeding condition. The aggregation remained compactly situated in a band a few to tens of meters wide and about 2 kilometers long, parallel to the shore. The band did not move appreciably during the observation period. The sea stars were feeding selectively on a coral which was a minor component of the total coral cover. The coral in the area was predominantly alive, and the proportion of dead coral did not increase appreciably during our period of observation.*

Since 1963 large aggregations of *Acanthaster planci*, the crown-of-thorns starfish, have been observed grazing on reef corals in the Pacific (*1, 2*). They were observed to be killing most of the hermatypic corals in several places along Australia's Great Barrier Reef and on the lee coast of Guam. In other places "dead" reefs are believed to have resulted from the feeding of *A. planci*.

In August 1969 a dense aggregation of *A. planci* was reported in the Kalohi Channel [8 miles (12.8 km) wide] off the south (lee) coast of the island of Molokai, Hawaii. This aggregation was featured in a documentary produced by a Honolulu TV station and has subsequently been investigated by the State Fish and Game Division and by a group of biologists from the University of Hawaii and the Bernice P. Bishop Museum. In April 1970, the State Fish and Game Division, in response to publicity about *A. planci*, attempted to eradicate the aggregation. Some pertinent observations were made during several months prior to and just after the attempted eradication.

Approximately 20,000 *A. planci* were aggregated in a band varying from a few to tens of meters wide and about 2 km long. The axis of the aggregation was oriented east and west, almost parallel to the coast and about 3 km offshore at depths varying from 12 to 30 m.

The bottom in the vicinity of the aggregation is covered with a dense uniform growth of coral, with occasional narrow sand channels running diagonally out from shore in a northeast-southwest direction. The coral cover is predominantly (about 90 percent) *Porites compressa*, a finger coral, extending about 1 m above the substrate. The second most abundant species (about 5 percent of the corals) is *Montipora verrucosa*, a sheetlike encrusting coral which usually occurs at the base of the *P. compressa* but occasionally grows over it to form larger colonies. This area of uniform coral cover is about 1 km wide and extends from near the 6-m contour to depths of about 30 m where the bottom becomes a sandy slope. It continues to the west of the aggregation for at least several kilometers. To the east of the aggregation, the coral cover is interrupted by a canyon 30 m deep. East of the canyon the uniform area of coral resumes and extends toward the end of the island. The head of the canyon is a steep slope. At depths of less than 20 m this slope is predominantly covered with *M. verrucosa*. At greater depths there is no coral cover.

In October 1969 a mile-long transect line approximately parallel to the aggregation was laid on the bottom by the State Fish and Game Division. This main east-west line was crossed every 250 yards (228 m) by lines extending north and south 250 yards on either side of it. The junctions and inshore ends of the lines were marked, both on the bottom with concrete blocks and on the surface with buoys. Five censuses of the sea stars were taken by the State Fish and Game Division at approximately 2-month intervals. Divers swam along these bottom lines, recording the numbers of *A. planci* within 10 yards on either side of 25-yard line segments. In April 1970 approximately 10,000 individuals were injected, each with 10 ml of household ammonia by means of hypodermic syringes, in an attempt to eradicate the aggregation. During the survey, from October 1969 to May 1970, the aggregation remained in the vicinity of the transects but moved up 55 m or less toward shore at the western end of the aggregation.

The aggregation was also sampled at about the same times by biologists from the University of Hawaii. *Acanthaster planci* were collected from the aggregation and examined aboard ship. Each animal was measured, weighed wet, and examined for sex and gonad state. Teams of divers also made estimates of species composition and the amount of dead coral along the transect lines. A few tagging experiments were also conducted.

The density of animals within the Molokai aggregation was variable. In one location 158 animals were collected from a circle of radius 10 m. The density was therefore one animal per 2 m². In other locations the animals were crowded together so that they often overlapped each other. Densely aggregated patches did not correspond with particular substrates. Some sea stars occurred on sand or coral rubble whereas others were found on live coral. The majority of the sea