sulfhydryl groups, can be oxidized even without urea during disc electrophoresis in persulfate-polymerized gels.

I recommend the use of riboflavin and light for polymerizing lower gels, rather than persulfate. The use of substances such as thioglycolate is of course restricted to proteins unaffected by these compounds.

JOHN M. BREWER
Department of Biochemistry, University of Georgia, Athens 30601

References and Notes

1. T. Jovin, A. Chrambaugh, M. A. Naughton, *Anal. Biochem.* **9**, 351 (1964).
2. B. G. Malmstrom, in *The Enzymes*, P. Boyer, H. Lardy, K. Myrbäck, Eds. (Academic Press, New York, 1961), vol. 5, p. 471.
3. J. M. Brewer and G. Weber, in preparation.
4. E. W. Westhead, *Biochemistry* **3**, 1062 (1964).
5. I thank Gene Michaels for preparing the photograph used as Fig. 1. Supported by NSF grant GB 5918.

14 February 1967

Temperature Tolerance of Some Antarctic Fishes

Abstract. *Three species of Antarctic fishes which live in constantly near-freezing waters have a markedly low upper-lethal temperature of 6°C ; this is the lowest upper-lethal temperature reported for any organism. The fishes survive supercooling to −2.5°C. Data on brain metabolism in vitro support the hypothesis that the central nervous system is a primary site of thermal injury.*

The temperature-tolerance limits of fishes and the physiological and biochemical factors setting these limits have been the subject of much study. Brett (*1*) reviewed the temperature-tolerance limits of a large number of fish species; Fry discussed the factors generally involved in creating these limits (*2*). Fry indicated that there is a lack of information about the temperature-tolerance limits of polar marine fishes which may spend their entire lives in near-freezing waters. There is still considerable uncertainty about the thermally sensitive site (or sites) which imposes thermal-tolerance limits on a species. This is particularly true in the case of the upper-lethal temperature which is often far lower than the temperatures at which enzyme denaturation is expected to occur.

We studied the temperature tolerance of several Antarctic fishes during an investigation of the cold-adaptation mechanisms of these organisms. The fishes used were three notothenioid species from McMurdo Sound (77°51′S, 166°38′E): *Trematomus bernacchii, Trematomus borchgrevinki,* and *Trematomus hansoni.* The family Nototheniidae occurs almost entirely in the Antarctic; the genus *Trematomus* is not known to occur in waters with temperatures higher than 1° to 2°C (*3*). The populations we sampled probably resided all year long in McMurdo Sound. Only *T. borchgrevinki* could not be captured throughout the year; its apparent disappearance during the three midwinter months may have been a consequence of our sampling procedures.

Table 1. Median resistance times for three Antarctic fishes adapted to -1.9°C. Time is given in minutes (1 week = 10,080 minutes). Numbers in parentheses represent numbers of specimens.

Species	Median time of death									
	15°C		10°C		8°C		7°C		5°C	
T. bernacchii	6	(6)	140	(12)	430	(8)			12,960	(6)
T. hansoni	8	(18)	60	(10)					15,840	(6)
T. borchgrevinki	7	(12)	81	(14)			1,905	(16)		

The temperature of the environment of these fishes is extremely cold and remarkably stable. The average temperature was −1.9°C; temperature variation, either with depth or through the year, was of the order of 0.1°C (*4*).

All specimens were captured through holes in the sea ice, with either wire traps or conventional hook-and-line fishing techniques (*5*). The fish were transported from the collecting sites to the biology laboratory at McMurdo Station in insulated containers; mortality during transit was negligible. Specimens were held, until use, in 190- or 760-liter aquariums which were continually aerated and filtered; the sea water was changed every 3 to 4 days. All species survived well under these holding conditions, and several specimens were held for periods in excess of 9 months with no apparent ill effects.

All experimental fish were held for 1 week or less at a temperature of −1.9° ± 0.1°C. There were not enough specimens and holding facilities available to permit the acclimation of groups of fish to different temperatures. We were therefore unable to establish a complete zone of tolerance (*6*) for the species. Specimen weights ranged from 34 to 177 g for each species. In view of the fact that most individuals arrived in the laboratory with full guts, no attempt was made to feed the specimens during this brief holding period. The photoperiod was regulated to match that of the environment, and, because most of the experiments were conducted during the austral winter, most of the specimens were held under conditions approximating total darkness.

In each experiment on lethal temperature, a group of four to ten fish of one species was placed in a 190-liter aquarium containing fresh, well-aerated sea water previously heated to the desired experimental temperature. Temperature variation was no greater than 0.1°C. The time of death was taken as the time at which opercular movement ceased and could not be reactivated by mechanically stimulating the specimen. Fish which had attained this state could not be revived if placed into cooler water. The highest temperature at which 50 percent of the fish survived for 1 week or longer is considered to be the upper incipient lethal temperature (*1*).

Determination of a lower incipient lethal temperature was not possible, due to our inability to supercool sea water to temperatures lower than −2.5°C. The longest period that we succeeded in keeping sea water this cold in an ice-free state was 3 days. Fish held in this supercooled water during this period showed no signs of distress; however, the appearance of ice crystals in the water led to rapid mortality, a phenomenon also observed with Arctic fishes by Scholander *et al* (*7*).

The lethal-temperature data (Table 1) indicate that all three species have a markedly low upper-lethal temperature. The upper incipient lethal temperature based on all data in Table 1 is approximately 6°C. We believe that this is the lowest upper-lethal temperature reported for any organism. The narrow thermal-tolerance range of these Antarctic fishes is consistent with the tendency (*1*) for the thermal-tolerance range to decrease as the adaptation temperature decreases.

The data on brain metabolism in

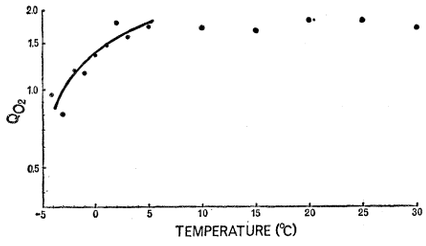

Fig. 1. Oxygen consumption in vitro of brain slices of *Trematomus bernacchii*.

vitro (Fig. 1) show an interesting relation to the lethal-temperature data. The oxygen consumption values were determined manometrically (*8*) with a medium described by Ekberg (*9*). After a fish had been killed by severing its spinal column immediately posterior to the brain, the roof of the skull was cut away, and the entire brain was removed and placed in chilled Ringer solution. After the meninges had been removed, the brain (excluding the hypophysis) was sliced into thin sections with a scissors. The tissues were immediately added to the manometer flasks that contained chilled medium. After a 15-minute period of temperature equilibration, readings were taken at a single temperature at 10-minute intervals for 3 hours. The metabolic rate. Q_{0_2}, based on the 1st hour of oxygen uptake, is expressed as microliters of O_2 per milligram of tissue (dry weight) per hour.

While it may be somewhat inaccurate to use data on brain metabolism in vitro as an index of the brain's ability to function adequately in vivo, the data (Fig. 1) nevertheless suggest that some type of breakdown occurred in the brain tissue at temperatures near the upper incipient lethal temperature. Extensive tissue disintegration in the Warburg flasks was also observed at temperatures of 5°C and higher. Studies of the metabolism in vitro of brain tissue of other fish species have yielded similar results (*10*). Wohlschlag (*5*) observed a decrease in the whole organism metabolism of *T. bernacchii* at temperatures of 1° to 3°C.

The metabolism in vitro of gill tissue of *T. bernacchii* did not exhibit this sharp sensitivity to temperature (*11*). This suggests that the thermal sensitivity of the central nervous system may be of primary importance in determining the thermal tolerance of the whole organism.

The site of thermal injury within the brain tissue cannot be conclusively determined from our data. However, the different sensitivities of brain and gill tissues to thermal injury and the low temperature at which impairment of brain metabolism occurred suggest that thermal death was not due to extensive enzyme denaturation. If direct thermal inactivation of one or more of the important cellular enzymes had been the result of exposure to the upper inicipient lethal temperature, an unlikely possibility at 5°C, then a similar pattern of inactivation would probably have occurred in both tissues. The rate of reaction of an isolated enzyme system from *T. bernacchii* actually increased linearly with temperature up to approximately 30°C (*11*). However, one cannot exclude the possibility that the brain possessed particularly thermolabile enzymes.

Nonenzymatic loci of thermal injury have been proposed. Heilbrunn (*12*) hypothesized that thermally caused changes in the biophysical state of the cellular lipids can lead to death. Study of the lipid composition of goldfish brain as a function of the acclimation temperature (*13*) supports Heilbrunn's hypothesis; it appears that the chemical properties of the brain lipids are adjusted during temperature acclimation to permit the maintenance of a constant "liquid crystalline" state of the cellular membranes (*13*). The temperatures at which this requisite biophysical state is disrupted may be considerably less extreme than the temperatures at which enzyme denaturation occurs. Thus, thermal death of the *Trematomus* fishes at 6°C may have resulted from subtle biophysical changes in the cellular lipids, perhaps the membrane lipids, rather than from direct thermal destruction of enzyme function.

GEORGE N. SOMERO
ARTHUR L. DEVRIES
Department of Biological Sciences,
Stanford University,
Stanford, California 94305

References and Notes

1. J. R. Brett, *Quart. Rev. Biol.* **31**, 75 (1956).
2. F. E. J. Fry, in *Handbook of Physiology*, Sec. 4, *Adaptation to the Environment*, D. B. Dill, Ed. (American Physiological Society, Washington, D.C., 1964), pp. 715–728.
3. J. R. Norman, in *Discovery Reports* (Cambridge Univ., London, 1940), vol. 18, pp. 1–104).
4. J. L. Littlepage, in *Biology of the Antarctic Seas*, M. O. Lee, Ed. (American Geophysical Union, Washington, D.C., 1965), vol. 2, p. 6.
5. D. E. Wohlschlag, in *Biology of the Antarctic Seas*, M. O. Lee, Ed. (American Geophysical Union, Washington, D.C., 1964), vol. 1, pp. 33–62.
6. F. E. J. Fry, *Publ. Ontario Fish. Res. Lab.* **68**, 1 (1947).
7. P. F. Scholander, L. van Dam, J. Kanwisher, T. Hammel, M. S. Gordon, *J. Cell. Comp. Physiol.* **49**, 5 (1957).
8. W. W. Umbreit, R. H. Burris, J. F. Stauffer, *Manometric Techniques* (Burgess, Minneapolis, 1964), pp. 1–17.
9. D. R. Ekberg, *Biol. Bull.* **114**, 308 (1958).
10. J. A. Freeman, *ibid.* **99**, 416 (1950); F. A. Fuhrman, N. Hollinger, J. M. Crismon, J. Field II, F. W. Weymouth, *Physiol. Zool.* **17**, 42 (1944).
11. G. N. Somero, unpublished.
12. L. V. Heilbrunn, *An Outline of General Physiology* (Saunders, Philadelphia, 1952), pp. 488–491.
13. P. V. Johnston and B. I. Roots, *Comp. Biochem. Physiol.* **11**, 303 (1964).
14. We thank members of the U.S. Navy Task Force 43, of the U.S. Naval Air Development Squadron Six, and of the U.S. Antarctic Research Program for their support and cooperation. This work was supported by NSF grants GA-45 and GA-537, administered by Drs. Donald E. Wohlschlag and Arthur C. Giese, respectively.

18 November 1966; 27 February 1967

Maternal and Environmental Influences on the Adrenocortical Response to Stress in Weanling Rats

Abstract. *Handling rat pups either for 10 or 20 days after birth resulted in a reduction of adrenocortical steroids in the plasma at weaning after the pups were exposed to novel stimuli as compared with controls that were not handled. Nonhandled offspring of mothers which had been handled in infancy also show a reduction in plasma steroids in response to novel stimuli when compared to nonhandled weanling rats of nonhandled mothers. Handling of the offspring when they are infants appears to counteract the influence of the experience of the mother during her infancy.*

Stimulation of an infant rat markedly affects the adrenocortical response to novel stimuli. Rats handled in their infancy from days 1 to 21 exhibit a significantly reduced steroid response after they are exposed to novel conditions in adulthood (*1*). Denenberg and Whimbey (*2*) report that, in rats, the offspring of mothers that had been handled in infancy were heavier at weaning as compared to offspring of mothers that had not been handled. Further, the experience of the mother during her infancy resulted in differential open-field behavior of the pups when they reached adulthood. The two experiments reported here were designed to investigate the question whether handling during infancy would affect the steroid response of pre-