COMPARATIVE BIOCHEMISTRY OF
ANTARCTIC PROTEINS

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The UCD team of agricultural scientists has been studying the proteins of Antarctic species for 11 years, starting in 1964. During the first seven years, trips were made each year to Antarctica to study and obtain specimens from penguins and cold-adapted fishes.

Prior to the start of the University's Antarctic program in the middle 1960's, our laboratory had long been studying the comparative biochemistry of proteins, particularly those of blood serum, milk, and egg whites. The main thrust of our studies was the use of differences in molecular structure of related proteins to help understand how the proteins function.

The Antarctic program started from a request by our laboratory to the U.S. Navy and the National Science Foundation for penguin eggs. We had found proteins in the eggs of other water fowl valuable to our studies on protein functions, and the penguin eggs seemed a logical choice for further study. The subsequent seven years' program of intensive work in Antarctica, as well as at our Davis laboratory, developed from observations made as our work progressed, and had the encouragement and financial and logistical support of the Biology Division of the Office of Polar Programs of the U.S. National Science Foundation. From 1964 to 1971, the senior author made six trips to Antarctica for periods varying from 3 to 9 weeks. The junior author and about 20 other students and associates all made at least one trip. Much of the work was done while living in fish houses on sea ice above a third of a mile of ocean water or in tent-huts surrounded by penguins. In addition to the scientific data we collected, one of the greatest returns was from the personal interrelationships of faculty, students, Navy personnel, and National Science Foundation employees. The overall story of the science and human relationships is de-

R. E. Fenney in foreground of living quarters (Jamesway tent) on slopes of Mt. Terror at Cape Crozier.

Group of researchers disembarking from plane on Ross Ice Shelf at McMurdo.

scribed in a current book, "Professor on the Ice" (Feeney, R. E., Pacific Portals, Box 490, Davis, CA 95616).

The work was separated into four closely integrated phases: 1) acquisition of specimens in Antarctica; 2) preparation and processing of samples in Antarctica, and the performance of all laboratory experimentation necessary to be done on fresh material; 3) transport of materials to Davis packed in ice, frozen, dried, or chemically purified; 4) long-term and more sophisticated laboratory work done at facilities of the University of California at Davis.

PENGUIN PROTEINS

Egg white proteins

A detailed study was first made of the egg white proteins of the Adelie penguin *(Pygoscelis adeliae)* see table 1.). With the Adelie egg white as an introductory standard, comparative studies were then made of egg white proteins of the Emperor *(Aptenodytes forsteri)*, Royal *(Eudyptes schlegeli)*, Yellow-eyed *(Megadyptses antipodes)*, Crested or Rockhopper *(Eudyptes crestatus)*, and White-flippered *(Eudyptula albosignata)* penguins. Eggs of the Adelie, Emperor, and Royal were obtained in Antarctica or on adjacent islands; the others were obtained from New Zealand.

In general, the properties of the egg white proteins of the different penguin species are remarkably similar to one another, compared to differences within other bird groups. The egg whites contain all of the homologous proteins found in the egg whites of other avian species. On a comparative basis, penguin egg whites are unusually high (approximately 5%) in sialic acid and unusually low (approximately 0.05%) in lysozyme. In addition, penguin egg whites are comparatively high in a protein (which our laboratory has named penalbumin) not normally detected in egg whites of many species.

In our studies, we found that penguin eggs deteriorate physically more rapidly than do chicken eggs when stored at 37°C. The mechanism of this important physical deterioration (weakening of the yolk membrane and thinning of the thick egg white) is not understood, but one theory holds that it is caused by interaction of egg white lysozyme with a gelatinous protein, ovomucin. Since penguin egg whites contain comparatively little lysozyme, our results indicate that the lysozyme theory is not tenable.

One of the penguin egg white proteins, ovomucoid, is like other avian ovomucoids in that it inhibits certain proteolytic enzymes. However, it is of importance in our work because it strongly inhibits the enzyme subtilisin. It has been a "standard" protein in our long-term researches on the biologically important inhibitors of proteolytic enzymes from beans, blood, milk, and chicken and turkey egg whites.

Blood serum proteins

Penguin blood serum proteins have general physical and chemical properties similar to those of blood serum proteins of other avian species. Electrophoretically, more differences were noted among blood proteins of penguin species than were noted among penguin egg white proteins. Also, the five isoprotein forms of the serum transferrin (iron-transporting protein), as seen electrophoretically, are similar to the five forms of the ovotransferrin in the egg whites. This was of particular interest in considering the genetic relationships of blood and egg white proteins, and the evolutionary significances of their five molecular forms.

FISH PROTEINS

Muscle and tissue enzymes

The functioning of organisms at low temperatures is important to many biological fields, including agriculture and medicine. Fish near the Ross Ice Shelf, McMurdo Sound, Antarctica, are exposed throughout the year to temperatures of -1.85°C, plus or minus only a few hundredths of a degree (with slightly larger variations depending upon the depths and the distances from the ice shelf). Our studies were primarily with two Antarctic fishes, *Trematomus borchgrevinki* and *Dissostichus mawsoni*. These were caught on hooks through holes in the ice, or were captured alive from seals. Six enzymes were studied: fructose-1,6-diphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase (GAPD), glycogen phosphorylas, lactic dehydrogenase (LDH), heart mitochondrial cytochrome oxidase system, and brain tissue acetylcholinesterase.

Adaptations of the enzymes to low temperature are given in table 2. In all instances, the enzymes functioned well at 0°C.

Blood proteins

The serum proteins of *T. borchgrevinki* and *D. mawsoni*, as well

TABLE 1. PROPERTIES AND CHARACTERISTICS OF THE MAIN CONSTITUENTS IN ADELIE PENGUIN AND CHICKEN EGG WHITES

CONSTITUENTS	GENERAL PROPERTIES	SPECIFIC CHARACTERISTICS	
		Chicken	Penguin
Ovalbumin	Has "masked" sulfhydryls; denatures easily	The major protein (~ 50%)	Smaller amount than in chicken
Penalbumin	Has active sulfhydryls, denatures easily	Absent	A major protein
Ovotransferrin	Complexes iron; antimicrobial	Has only 2 forms (isoproteins)	Has 5 forms (isoproteins)
Ovomucoid	Inhibits proteolytic enzymes	Inhibits trypsin	Inhibits trypsin at 1 site; inhibits α-chymotrypsin & subtilisin competitively at another site
Ovomucin	Viscous, responsible for gel of thick egg white	A minor protein	No differences from chicken noted
Lysozyme	Hydrolyzes polysaccharides, antimicrobial	Moderate concentration (3.2%)	Very low concentration ($\leq$ 0.02%)
Ovomacroglobulin	Strongly antigenic	A minor protein	Twice the concentration present in chicken
Flavoprotein	Binds riboflavin	A minor protein	Same as in chicken, but no riboflavin bound to it
Avidin	Binds biotin; antimicrobial	A minor protein	Has several forms (isoproteins)
Sialic Acid	Acidic compound bound to proteins	Relatively small concentration (0.3%)	Very high concentration (~ 5%)

TABLE 2 COMPARATIVE EFFECTS OF TEMPERATURE ON ENZYMES

Enzyme	Differences between enzymes from cold-adapted fish and those from warm-adapted species*
Muscle Aldolase	Fish enzyme much more labile to warm temperatures and to sulfhydryl reagents
Muscle Glyceraldehyde-3-phosphate Dehydrogenase	Fish enzyme had lower activation energy and thus was comparatively more active at low temperatures. Warm species enzyme inhibited by nucleotides at low temperature-but fish muscle more resistant
Muscle Glycogen Phosphorylase	Enzymes similar
Muscle Lactic Dehydrogenase	Fish enzyme had lower activation energy and thus was comparatively more active at low temperatures
Heart Mitochondrial Oxidase System	Fish system had same activation energy from 0°C to 30°C. Warm species system had large change (increase) when cooled below 20°C, possibly due to changes in lipids
Brain Acetylcholinesterase	Enzymes similar

*Warm-adapted species usually the rabbit, but also seal and cow

as of several other Antarctic fishes, were compared electrophoretically. Differences in the patterns were easily discernable, and in some instances large. In our initial studies we reported on a pronounced adaptation of the blood clotting system for efficient function at -2°C. Later studies have concentrated on an antifreeze glycoprotein in the blood serum. A substance in the blood of Arctic fishes which abnormally lowers the freezing point was first noted by Per Scholander (Professor Emeritus, UCSD) and co-workers. A. L. DeVries, a former member of our team, and now at UCSD, and D. E. Wohlschlag, also found such a substance in Antarctic fishes, and determined that it was a glycoprotein with a very simple structure containing only two amino acids — alanine and threonine. The physical and chemical properties of the antifreeze glycoprotein have been studied in detail in our laboratory.

Antifreeze glycoprotein is unique in several respects. For example, it is composed of repeating units of a glycotripeptide, alanine, alanine, threonine, with each threonine glycosidically linked to a disaccharide. Molecular weights of the active components vary from approximately 11,000 g to 25,000 g. The glycoprotein also lowers the freezing temperature of water more than twice as much as does an equal weight of NaCl. Another significant characteristic is that, although it lowers the freezing temperature, it has no effect on the melting temperature, and thus does not function by colligative properties. In other words, the fish can only tolerate osmotically enough low molecular weight substances to lower the freezing temperature to approximately -1.1°C to -1.2°C. The antifreeze glycoprotein lowers the freezing temperature the remainder of the way (to lower than -1.9°C), and it does this without further affecting the osmotic pressure, which would be lethal to the fish.

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R. E. Feeney at Cape Crozier in rookery of 300,000 Adelie penguins.