Sea Anemone Neuromuscular Responses in Anaerobic Conditions

Abstract. *The sea anemone* Bunodosoma cavernata *survives anoxic conditions for as long as 6 weeks. Whether or not oxygen is present, its neuromuscular responses are the same and the pulses of its nerve net have the same threshold size and shape.* Bunodosoma cavernata *buried in the sand during a low tide are exposed to anoxic conditions. Their neuromuscular responses in anaerobic conditions ensure protective withdrawal behavior should the need arise.*

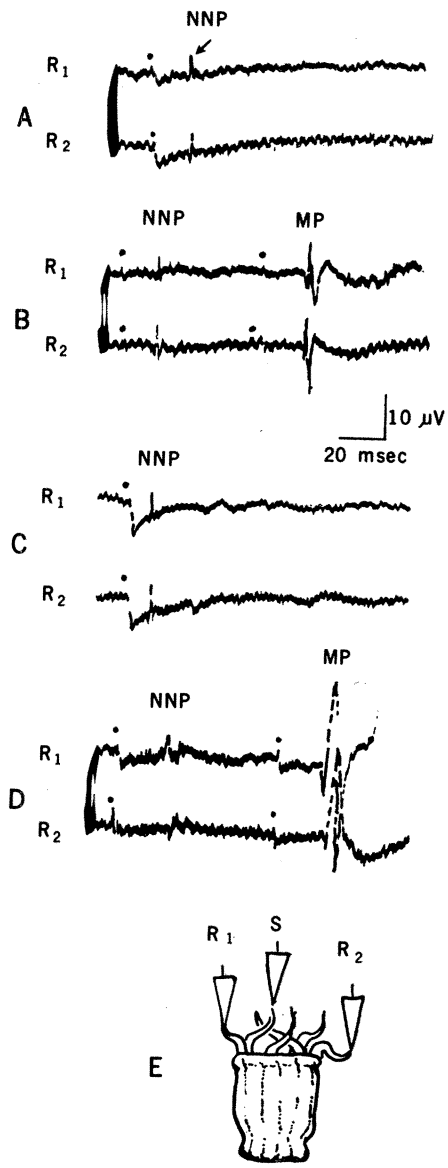
Facultative anaerobic invertebrates occur in a number of phyla. Distinctive pathways of intermediary metabolism in anaerobic conditions have been described in several of these phyla (*1*). Sea anemones have been reported to have various degrees of anaerobic capabilities (*2*). This report describes a common Texas Gulf Coast sea anemone that survives long-term anoxia and shows that the animal's neuromuscular system continues to function normally during the period of anaerobic metabolism.

Jetties and groins are the only rocky substrate for marine organisms along the Texas Gulf Coast. The sea anemone *Bunodosoma cavernata* is a common member of the jetty community. The anemones live attached to the rocks in the middle and lower intertidal zone. Often the columns of these anemones are buried in the sand with only the tentacles exposed, and some of the anemones are found underneath rocks where the sand is black from anaerobic sulfur bacteria.

Previous studies showed that anemones in environments with very little oxygen switch to anaerobic metabolic pathways (*3*). We undertook to ascertain the survival of the jetty anemones in anoxic conditions. Anemones were collected from exposed granite rocks of the jetties at Galveston, Texas, and were maintained in a seawater aquarium before the experiments. The animals were placed individually in sealed chambers containing seawater that had been flushed with nitrogen. As determined with a polarographic oxygen electrode and by the Winkler method, the oxygen concentration in the water was less than 0.1 part per million (ppm) at the beginning of the experiment. After a few hours in this anoxic condition, the anemones withdrew their tentacles and contracted the column. They were left alone for 6 weeks, and then the jars were opened, releasing a pungent odor of sulfur. There was no measurable oxygen left in the water. The anemones from the sealed chambers were now placed in aerated water. All opened quickly, expanding the column, disc, and tentacles. Thus all the anoxic anemones survived, as did the aerated controls.

C. Sassaman and C. P. Mangum (*3*) reported that *Haloclava producta* survived for 11 days in water with less than 0.5 ppm of oxygen by shifting to anaerobic metabolism. However, survival for 6 weeks in anoxic conditions at room temperature is rare among free-living animals, regardless of phylum.

Since the anemones withstood such long periods of anoxia, it was appropriate to see whether their neurophysiological responses were the same during anaerobic and aerobic metabolism. In aerobic conditions, the jetty anemone withdraws its tentacles and shortens in response to four or five suprathreshold electrical stimuli delivered less than 1 second apart (*4*). These stimuli activate the nerve net of the retractor muscles in the column of the anemone. This nerve net is through-conducting, but facilitation is required at the neuromuscular junctions before a fast withdrawal response can occur.



To see whether their neuromuscular system would continue to function in anaerobic conditions, jetty anemones that had been kept anaerobic for 48 hours were placed in seawater chambers with covers with small openings that permitted the passage of nitrogen gas tubing, stimulating electrode, oxygen probe, and thread from the anemone column to the myograph of a Narco Physiograph. Nitrogen was bubbled through the chamber continuously, keeping the oxygen level below 0.1 ppm.

In the anoxic environment each anemone responded normally to the electrical stimuli. There was no response to the first suprathreshold electrical stimulus and a slight contraction in response to the next stimulus if delivered within 1 second. Four or five consecutive stimuli resulted in the complete contraction of the anemone, which would already have partly withdrawn its tentacles. Thus the animal's neuromuscular system continued to function normally without oxygen. Metabolic inhibitors also provided interesting results. In seawater containing 0.01*M* KCN, anemones survived for several days and responded normally to electrical stimuli. When transferred back to the aquarium, they showed no ill effects. However, when placed in seawater containing 0.001 percent iodoacetate, a glycolysis inhibitor, they died in a few hours.

To determine whether the nerve net functions normally under anaerobic conditions, electrical pulses associated with the net were recorded with suction electrodes after the method devised by Josephson (*5*). The signal was amplified by a differential preamplifier, displayed on an oscilloscope, and recorded photo-

Fig. 1. Electrical activity recorded from two tentacles of *B. cavernata*. (A and B) Recordings in aerobic conditions. (C and D) Recordings in anaerobic conditions; 48-hour exposure. (A) and (C) show NNP's in response to single suprathreshold stimuli (dots). (B) and (D) show NNP's and muscle potentials (*MP*'s) in response to two suprathreshold stimuli less than 1 second apart. The NNP in response to the second shock just precedes and is hidden by the muscle potential. (E) Recording electrodes (R_1, R_2) and stimulating electrodes (*S*) on the anemone.

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graphically. Electrical stimulation was administered through another suction electrode. Although McFarlane (*6*) described a number of conducting systems for anemones, in our study only the nerve net pulses (NNP's) and the potentials of the retractor muscle were examined (*7*).

Preliminary studies of anemones in aerobic conditions revealed NNP's of 10 μV or less (Fig. 1A). Threshold was 5 to 7 V when a 1-msec stimulus was given. Anemones that had been kept in sealed anaerobic chambers for 1 week were placed in recording chambers with nitrogen bubbling through the seawater; as before, the oxygen levels were less than 0.1 ppm. The contracted posture of the animals made it difficult to attach the suction electrodes; however, one or two tentacles were usually exposed.

The NNP's recorded from the anoxic anemones were identical in configuration and threshold to NNP's seen in anemones in aerobic conditions (Fig. 1C). Also, two shocks 0.5 second apart induced the muscle potential that is usually associated with the second stimulus (Fig. 1, B and D). Thus the neuromuscular system does not fatigue any more rapidly in anaerobic conditions.

It is generally accepted that nerve cells require oxygen to function. The sodium ion pump requires adenosine triphosphate (ATP) generated by the electron transport system. One well-known exception is found in the sea turtle. The central nervous system of this animal functions anaerobically for hours during dives (*8*). Glycolysis is thought to generate sufficient ATP for the turtle to survive. To my knowledge, the anaerobic function of the nervous system of a faculative anaerobic invertebrate has not been reported until now. The *B. cavernata* nerve net and retractor muscle function normally in the absence of oxygen or in the presence of the cytochrome inhibitor KCN. There is sufficient ATP stored or, more likely, produced by anaerobic metabolic pathways for an anemone to execute a protective withdrawal response in anaerobic conditions. Invertebrates that survive anoxic environments maintain important behavioral and physiological functions despite lower production of ATP.

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References and Notes

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2. C. Sassaman and C. P. Mangum, *Comp. Biochem. Physiol. A* **44**, 1313 (1973).
3. ______, *Biol. Bull. (Woods Hole, Mass.)* **143**, 657 (1972).
4. C. F. A. Pantin, *J. Exp. Biol.* **12**, 119 (1935). The early studies by Pantin of the sea anemone nervous system analyzed the quick withdrawal response. This response is a rapid and symmetrical contraction primarily involving the retractor muscle on the mesenteries. The movements were recorded on a kymograph. The nature of the conduction mechanisms was inferred from the relation between controlled electrical stimuli and the resulting contraction. Direct study of the nervous system was difficult because the anemone has a diffuse nerve net. Large bipolar cells in the retractor muscle are 4 to 8 mm long but usually not more than 2 μm in width, and they can be seen only with careful staining. The quick withdrawal response to controlled electrical stimuli still provides a fast method for determining the integrity of the sea anemone nervous system.
5. R. K. Josephson, *J. Exp. Biol.* **45**, 305 (1966). Electrical potentials can be recorded from the tentacles with suction electrodes. Recording and stimulation electrodes are made from drawn plastic tubing with an internal diameter of 0.2 to 0.4 mm. The plastic tubing is attached to a syringe and the electrode is held on the tentacle with a slight suction; electrical contact is made with chlorided silver wires in the plastic tubing. The electrode is filled with seawater by withdrawing the syringe plunger. (Chlorided silver wires placed in the seawater surrounding the preparation provide indifferent electrodes.) The potentials are amplified and displayed on an oscilloscope. Electrical stimuli are low-voltage pulses 1 msec in duration.
6. I. D. McFarlane, *J. Exp. Biol.* **51**, 377 (1969).
7. P. E. Pickens, *ibid.*, p. 513. Suprathreshold stimuli can evoke two kinds of electrical potentials associated with the rapid contraction or through-conduction system. These are (i) small NNP's that are all-or-none and of short duration and (ii) large muscle potentials.
8. P. W. Hochachka, in *Comparative Animal Physiology*, C. L. Prosser, Ed. (Saunders, Philadelphia, 1973), p. 227.
9. I thank P. E. Pickens for his help and for the use of his research facilities in the Department of Cellular and Developmental Biology, University of Arizona. Funding was provided by the Organized Research Fund of Texas A & M University.

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Antibody to Spermine: A Natural Biological Constituent

Abstract. *A protein that binds spermine specifically was separated from normal rabbit serum by affinity chromatography. Immunoelectrophoresis, the Ouchterlony immunodiffusion test, and gradient gel electrophoresis indicated that this protein has immunoglobulin characteristics and consists of several populations of antibodies to spermine. These were sequentially released from Sepharose-spermine gel by stepwise elution with solutions ranging in* pH *from 4 to 1. The binding constants varied from* 5.0×10^8 *to* 11.1×10^8 *liters per mole. These globulins did not react with monoacetylputrescine,* L*-ornithine,* L*-lysine, and histamine. Negligible cross-reactivity was detected with spermidine, putrescine,* N^8*-monoacetylspermidine, cadaverine, and diaminopropane. Since perturbations in polyamine metabolism have been identified in several diseases, the study of extracellular polyamine homeostasis may reveal an important regulatory function for this protein.*

Intracellular aliphatic polyamines and diamines in micromolar quantities facilitate tissue growth, hypertrophy, and regeneration (*1-5*). By contrast, no more than picomolar levels of extracellular polyamines (free, acetylated, and bound) are observed normally (*6-10*). In a number of systems, extracellular free polyamines may promote or inhibit cell growth depending on their concentration (*11-15*). Body polyamine homeostasis may be seriously disturbed under conditions of uncontrolled production, tissue damage, or renal retention (*16-18*). These organic cations may bind nonspecifically or specifically to cell membrane components (*19, 20*). Similar intracellular interactions have been described for proteins, nucleic acids, and cell organelles (*21-23*).

During the purification of induced rabbit antibodies to spermine with affinity chromatography (*24*), we noted that control rabbit serum treated in the same way as serum from immunized animals contained a protein fraction with strong binding affinity for spermine. As a consequence, we completed the separation and characterization of this active

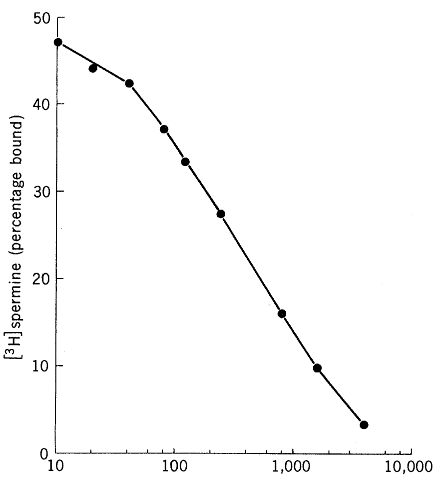

Fig. 1. Inhibition curve for isolated anti-spermine antibody (pool 4). Thirty thousand DPM of [^{3}H]spermine was used per assay test tube (incubation volume, 1 ml). Binding at 0 pg of unlabeled spermine was 48 percent; at 10 pg, 47.2 percent. Charcoal dextran solution (0.1 ml per assay test tube) (*38*) was used for separating bound and free spermine. After being mixed and incubated at 4°C for 20 minutes, the charcoal was centrifuged at 3000 rev/min for 25 minutes in radioimmunoassay test-tube carriers in a Beckman J-6B refrigerated centrifuge; the supernatant fraction, containing bound ligand, was then counted.