(7), cesium applied internally (8), sodium fluoride perfused internally (9), internal media of low ionic strength (10), and scorpion venom (11). However, some difference is noted between CTX and the other agents. For the other agents, it was concluded that the magnitude of the steady-state potassium current was decreased along with the prolongation of the early transient current. This may suggest a coupling between these two processes. It remains to be seen whether CTX is consistent in its lack of effect on the potassium channel and therefore different from the other agents in its action on the possible coupling process.

Condylactis toxin is a rather large molecule (molecular weight 10,000 to 15,000) (12), and it is interesting to see how such a large molecule specifically slows the turn-off without affecting the turn-on of the early transient conductance. Because CTX did not affect the turn-off of the transient conductance in squid giant axons, there may be some species specificity of the CTX action, requiring a special configuration of membrane molecular structure.

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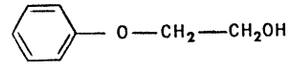
Phenoxyethanol: Protein Preservative for Taxonomists

Abstract. *Pieces of chicken heart or skeletal muscle were placed in a dilute solution of the antimicrobial agent 2-phenoxyethanol and stored at room temperature. Under these conditions, the serum albumin, lactate dehydrogenase, and malate dehydrogenase in these tissues survived in easily detectable amounts for at least 2 weeks. The surviving proteins appeared to be identical with those of fresh tissues in physical, catalytic, and immunological properties. Phenoxyethanol also preserved heart and muscle proteins of representatives of other vertebrate classes. Tissue samples collected in the field may be thus preserved for later analysis by biochemical taxonomists.*

Biochemical methods are used more and more to classify species (1) and elucidate their evolutionary history (2). To simplify the collection and transportation of samples collected in the field, we sought a preservative that prevents bacterial and fungal growth and yet permits the survival of native proteins at room temperature for several weeks (3).

The compound 2-phenoxyethanol, also known as phenoxetol or ethylene glycol monophenyl ether, appears to be such a preservative.



It is a colorless, nonvolatile, nonflammable, stable, and nearly odorless liquid. Moreover, it is low in price and relatively nontoxic. Phenoxyethanol has received limited clinical and industrial use both as an antibacterial and antifungal agent (4). It has been employed at the British Museum as a preservative for animals and plants, after fixation with formaldehyde or Bouin's fluid, but invertebrates that have not been first fixed in formaldehyde disintegrate within a few weeks when stored in 2-phenoxyethanol (5). Thus, to be of use to the biochemical taxonomist, selected tissues and not whole organisms have to be placed in the 2-phenoxyethanol solution.

The preservative properties of phenoxyethanol are illustrated by our experiment with chicken tissues. A piece of heart muscle (1 g) was put in 5 ml of a 2 percent (by volume) solution of phenoxyethanol in distilled water and stored in a screw-capped glass vial (15-ml capacity) at room temperature (22° to 28°C) on a laboratory bench. After 2 weeks, the contents of the vial were homogenized. The homogenate was clarified by centrifugation at 30,000*g* for 10 minutes. Some of the resulting supernatant fluid was assayed spectrophotometrically (6, 7) for lactate dehydrogenase activity. The total number of enzyme units (8) in the vial was calculated to be 600; in comparison, 1 g of fresh heart muscle contained about 650 units of lactate dehydrogenase activity.

With crystalline chicken H_4-lactate dehydrogenase (9) and a freshly prepared extract of chicken heart muscle as controls, we examined the following properties of the surviving lactate dehydrogenase: (i) electrophoretic mobility in starch gel at *p*H 7; (ii) sensitivity to thermal denaturation under standard conditions; (iii) Michaelis constant (K_m); (iv) sensitivity to inhibition by pyruvate; and (v) reactivity in immunodiffusion and microcomplement fixation tests with rabbit antiserum directed against pure chicken H_4-lactate dehydrogenase (Fig. 1A). These properties of the fresh enzyme have been described (6). The enzyme from the phenoxyethanol-treated tissue appeared identical with the pure enzyme and with that from fresh tissues.

A similar experiment was conducted with a 1-g portion of chicken breast muscle, which contains M_4-lactate dehydrogenase, an enzyme that is quite distinct chemically from the H_4 enzyme (6, 9). After 2 weeks, all of the original M_4 enzyme activity was still present. The surviving enzyme had normal electrophoretic properties.

The two isoenzymes of malate dehydrogenase also survived in these experiments with chicken heart and breast muscle. The recovery of malate dehydrogenase activity at 2 weeks was 20 percent. Electrophoretic analysis revealed that the mitochondrial isoenzyme survived to a lesser extent than did the supernatant isoenzyme. The mobilities of the surviving isoenzymes were normal (10).

Serum albumin, which occurs in readily detectable amounts in excised muscle tissues, also survived in the above experiments. This protein was detected by electrophoresis as well as by immunological tests with rabbit

antiserum directed against purified chicken serum albumin (*11*). The phenoxyethanol-treated material could not be distinguished from untreated chicken serum albumin by means of the immunodiffusion test or by the more sensitive microcomplement fixation test (Fig. 1B) (*12*).

The above proteins leaked out of the tissue into the surrounding phenoxyethanol-containing fluid, and, after 2 weeks at room temperature, only 10 to 15 percent of the surviving lactate and malate dehydrogenase activities remained in the tissue. Since the stored tissue retained its original shape and 95 percent of its original wet weight, the leakage of proteins was probably selective. Presumably, contractile and structural proteins which account for much of the organic matter in muscle did not leak from the tissue.

These findings were not unique to chicken tissues. Similar experiments were conducted with heart and skeletal muscle samples from a laboratory rabbit (*Oryctolagus cuniculus*), a lizard (*Iguana iguana*), and a salmon (*Oncorhynchus tshawytsha*). Lactate and malate dehydrogenase activity survived to an appreciable extent when these tissue samples were stored for 1 week at room temperature, although the survival of lactate dehydrogenase in salmon heart treated with 1 or 2 percent phenoxyethanol was poor.

Samples from the field have been stored in phenoxyethanol. Heart and breast muscle samples of a charadriiform bird (*13*) were collected in Chile, preserved as described in 2 percent phenoxyethanol, and sent without refrigeration by airmail to Berkeley, arriving 9 days later. Both H_4 and M_4 isoenzymes of lactate dehydrogenase, as well as the mitochondrial and supernatant malate dehydrogenases, were detected in the fluid surrounding the tissues by spectrophotometric assay and gel electrophoresis. The supernatant malate dehydrogenase had the unusual electrophoretic mobility that is characteristic of this enzyme in birds of the order Charadriiformes (*10*). Phenoxyethanol also received extensive use this summer on a lizard-collecting expedition in South America and the Caribbean area. The enzymes H_4-lactate dehydrogenase and supernatant malate dehydrogenase survived well in phenoxyethanol-treated heart samples from various species of lizard.

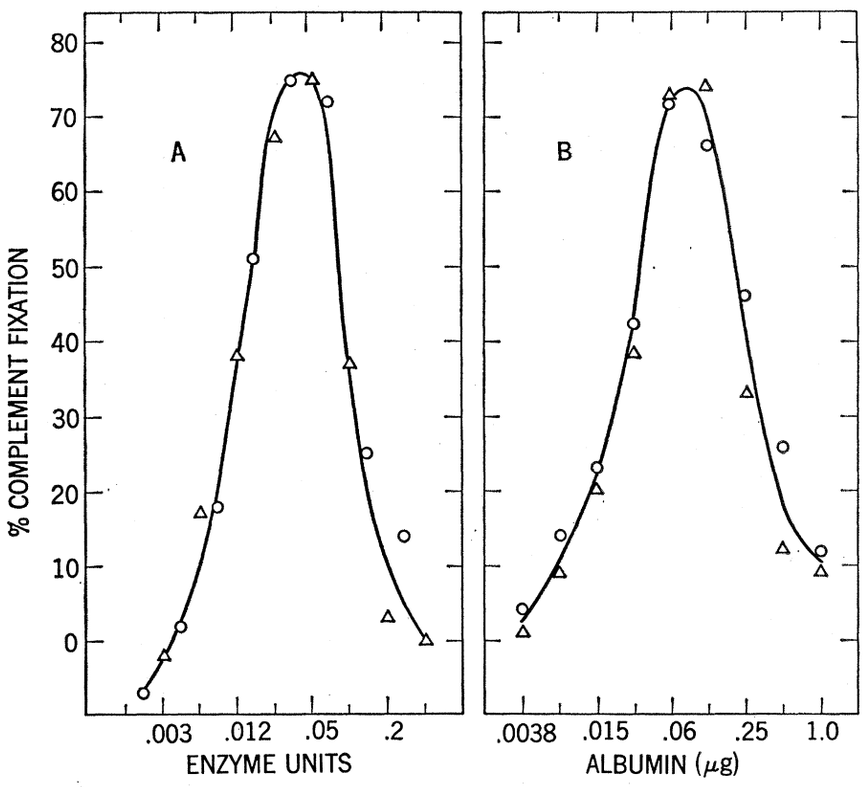


Fig. 1. Quantitative immunological comparison of native chicken proteins with those from a 1-gram piece of chicken heart muscle stored for 2 weeks at room temperature in 5 ml of a 2 percent solution of phenoxyethanol. Serial dilutions of the phenoxyethanol-treated sample (△) and a fresh chicken heart extract (○) were tested for reactivity with two specific rabbit antiserums. Reactivity was measured by the quantitative microcomplement fixation method (*21*). Antiserum 174B5, which was used to obtain the curves on the left (A), was directed against pure chicken H_4-lactate dehydrogenase (*6*). Antiserum 7B1, which was used to obtain the curves on the right (B), was directed against pure chicken serum albumin (*11*). A curve identical in height and shape to that in Fig. 1B was given by pure chicken serum albumin. The quantity of albumin in the heart samples was calculated from the fact that the quantity of pure chicken albumin required to produce maximum fixation of complement is 0.09 μg. Thus chicken heart muscle, according to our calculations, contains approximately 1.5 mg of albumin per gram (wet weight) (*22*).

At first it seems remarkable that native enzymes and other globular proteins survive so well in dead biological material at room temperature (*14*). Peptidases are known to occur in heart and skeletal muscle (*15*) and presumably are responsible for in vivo degradation of muscle proteins. Since the half-life of muscle proteins in vivo is on the order of weeks or months (*16*), the activity of proteolytic enzymes in these tissues must be very low.

Undoubtedly some proteins will fail to survive for 2 weeks at room temperature in phenoxyethanol-treated tissues. Proteins would be expected to break down rapidly in spleen, liver, kidney, and intestinal mucosa because such tissues contain large numbers of lysosomes. In addition, proteins are more sensitive to thermal denaturation in the presence of phenoxyethanol (*17*).

Consequently, only those proteins which are rather resistant to thermal denaturation will survive for long periods in phenoxyethanol solutions at room temperature. Mitochondrial malate dehydrogenase, for example, is rather sensitive to thermal denaturation and, accordingly, it survived poorly in phenoxyethanol-treated muscle samples. Its survival was enhanced by including sucrose in the preserving fluid. Sucrose has also been found to protect other proteins against denaturation (*18*). Hence it appears desirable to include sucrose (0.25*M*) along with phenoxyethanol in preserving fluids for unstable proteins.

We want to emphasize the value of phenoxyethanol for field work. Twenty ml of 2-phenoxyethanol is enough for making up 1 liter of preserving fluid; only water need be added (*19*). Rea-

gents like 2-phenoxyethanol make cooling facilities unnecessary in the field or during transportation to the laboratory (*20*).

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7. Phenoxyethanol, even at a final concentration of 2 percent in the reaction mixture, does not interfere with the enzyme assay.
8. An enzyme unit is defined as that amount of lactate dehydrogenase which produces an absorbance change of 1.0 per minute at 340 nm in a 3-ml reaction volume at 23°C and is approximately equal to 1 μg of lactate dehydrogenase or 1 international unit (at 30°C).
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12. After storage of chicken tissues for 1 year in 2 percent phenoxyethanol at room temperature, H_4-lactate dehydrogenase and serum albumin were still readily detectable but less than 1 percent of the original malate dehydrogenase and M_4-lactate dehydrogenase remained. Moreover, the surviving lactate dehydrogenase and serum albumin were now slightly modified in their electrophoretic and immunological properties. There was no gross evidence of microbial contamination, and, except for slight discoloration, the tissue appeared normal. After a year, the tissue weighed 95 percent of the starting value, indicating that little autolysis had occurred.
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Fluorescence Changes during Conduction in Nerves Stained with Acridine Orange

Abstract. *Nerves from spider crabs and squid fluoresce when stained with Acridine Orange. The intensity of fluorescence increases during nerve conduction. Prolongation of the electric response in the squid axon is associated with a fluorescence change of similar duration. These findings suggest that the physicochemical properties of the macromolecules around the dye molecules in the nerve membrane drastically change during the process of nerve conduction.*

The process of conduction of a nerve impulse is considered by several investigators (*1*) to involve a reversible cooperative change in conformation of the macromolecules in the nerve membrane. Recent studies of various optical properties of nerves (*2*) support the view that some kind of conformational change does take place in the membrane during conduction. This report presents our findings that the fluorescence yield of a nerve stained with Acridine Orange (AO) changes during nerve conduction, and suggests that this change is a sign of a conformational change of the membrane macromolecules.

Optical properties of complexes of AO with polynucleic acids, polypeptides, and other macromolecules have been studied as a means of elucidating the secondary and tertiary structure of the macromolecules (*3–5*). Variations in the absorption and emission spectra of AO-macromolecule complexes have been analyzed on a statistical and quantum-mechanical basis (*5*). Acridine Orange has also been used as a vital stain for nerve cells and fibers (*6*). Many biologists believe that the fluorescence of tissues stained with AO is a sensitive indicator of the state (or "vitality") of the cells (*7*). For these reasons, a study of the changes in fluorescence of the AO-membrane complex is expected to yield significant information concerning the state of the membrane macromolecules during nerve conduction.

Nerves taken from spider crabs (*Libinia emarginata*) and from North Atlantic squid (*Loligo pealei*) were used in the present studies. The dye, AO, was dissolved in either artificial seawater (for external application) or a potassium phosphate–glycerol solution containing 300 meq of K^+ per liter (for internal application). The concentration of AO was between 0.05 and 0.1 mg/ml. Crab nerve fibers were stained by immersion of a 5- to 8-mm portion of the nerve in the AO solution for approximately 10 minutes and subsequently washed with seawater free of dye. Intracellular application of AO was done by perfusing the interior of a 4-mm-long portion of a squid giant axon with the AO-containing solution for a period of 5 to 10 minutes; the AO solution was kept in the axon interior during the fluorescence measurements. The technique of intracellular perfusion has been described previously (*8*).

A stained nerve was mounted in a chamber (made of black lucite) filled with artificial seawater (see Fig. 1, top). The chamber was provided with two pairs of platinum electrodes. One pair of the electrodes was used to