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THE EFFECT OF ACID WATER ON THE LOSS OF DIVALENT CATIONS AND PRIMARY AMINES FROM NATURAL MEMBRANES

JAMES H. SWINEHART and MARCOS A. CHENEY
Department of Chemistry, University of California, Davis, CA 95616, USA

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Abstract—1. Losses of Ca^{2+}, Mg^{2+} and primary amines into waters between pH = 5 and 3 from eyed Chinook salmon eggs, *Oncorhynchus tshawytscha*, and gills excised from the freshwater, bivalve mollusc *Anodonta californiensis* were measured and compared to effluxes into distilled water.

2. Sulfuric, nitric and hydrochloric acids were used.

3. Even at pH = 5 losses of Ca^{2+} and Mg^{2+} from both biological systems occur at short times, minutes, and can exceed those found in water of higher pH (non-acid waters).

4. Increasing acidity increases short term primary amine loss from both systems.

5. For both divalent cation and amino acid losses gills of *A. californiensis* are more sensitive to acidity than eggs of *O. tshawytscha*.

INTRODUCTION

Much has been written about acid rain both in the scientific and popular literature (La Bastille, 1981; Likens *et al.*, 1979; Hutchison and Hayas, 1978; Toribara *et al.*, 1980). It is clear from all accounts that acid rain, which evolves from the dissolution of oxides of sulfur and nitrogen in atmospheric water, and the waters of lakes and streams resulting from the rains can do irreversible damage to living organisms. There are two chemical modes of action of these acid waters. First, the acid water can act directly on the organism. Second, acid water can act indirectly by mobilizing detrimental cations and anions in the soil, stream and lake sediments (Schindler *et al.*, 1980), and particulates on the surface of an organism (Lindberg *et al.*, 1982).

The purpose of this work is to study the mechanisms by which acid waters at pH = 5.0, 4.0 and 3.0 interact with the membranes of two organisms: eyed Chinook salmon eggs, *Oncorhynchus tshawytscha*, and the excised gills of the freshwater, bivalve mollusc *Anodonta californiensis*. The acids used in this study are sulfuric and nitric acids (the constituents of acid rain) and hydrochloric acid. The thrust of the work centers on the effects of these acids on the losses of Ca^{2+} and Mg^{2+}, two divalent cations important to the integrity of the membrane and the functioning of membrane processes, and primary amines, which are contained within the organism. A study of the leakage of primary amines from these organisms serves as a measure of the effect of acid on the integrity of the membranes.

MATERIALS AND METHODS

Specimens and solutions

Specimens of the freshwater, bivalve mollusc *A. californiensis* were collected in irrigation canals approx. 5 miles north of Knights Landing, California. Specimens were used within a week of collection and were maintained in an aquarium having water and temperature conditions of the natural environment. Eyed Chinook salmon eggs, *O. tshawytscha*, were obtained from the California Department Fish and Game Commission fish hatchery on the American River in Rancho Cordova, California. The eggs were stored for short times in plastic containers containing water from the American River, which was aerated and cooled. The eggs were used within a few days after they were obtained from the fish hatchery, and the mortality rate, as evidenced by an opaque appearance of the egg, was low. Eggs showing the slightest opaqueness were discarded.

The solutions used in the experiments were prepared from distilled water (W) which had a pH of 5.6–6.0 due to the dissolution of atmospheric carbon dioxide. The pH of a solution was adjusted by the addition of reagent grade sulfuric, nitric or hydrochloric acid and the pH was measured with an expanded scale pH meter which had been calibrated with standard buffers.

Divalent cation and primary amine loss experiments

The aim of these experiments was to determine the loss of Ca^{2+}, Mg^{2+} and primary amines from fish eggs and excised gill from *A. californiensis* as a function of the type of acid and varying acidity. In preparation for the experiment the fish eggs were washed in unchlorinated water and then briefly in distilled water, drained, dried lightly and used immediately. The gills of *A. californiensis* were excised, placed in unchlorinated water, and allowed to incubate for 30 min to facilitate recovery of gill cilia activity. The gills were washed briefly in distilled water, drained, dried lightly, and used immediately.

When an experiment was initiated, the fish eggs (20) or gills (0.10–0.15 g dry weight) were placed in 200 ml of the appropriate solution in a plastic beaker. Stirring was achieved with a shaker table. The pH was kept constant by the periodic addition of small amounts of the appropriate acid. Two milliliter samples of the solutions were taken periodically. Analyses for Ca^{2+} and Mg^{2+} were carried out using standard atomic absorption methods with an Instrumentation Laboratory Model 751 Atomic Absorption Spectrometer, and primary amines were determined by the fluorescamine method (Udenfriend *et al.*, 1972). The concentrations of the primary amines were calculated by comparison with the fluorescence of a glycine standard and results were expressed as "glycine equivalents" (Crowe *et al.*, 1977; Stephens, 1975; Wright and Stephens, 1977). The

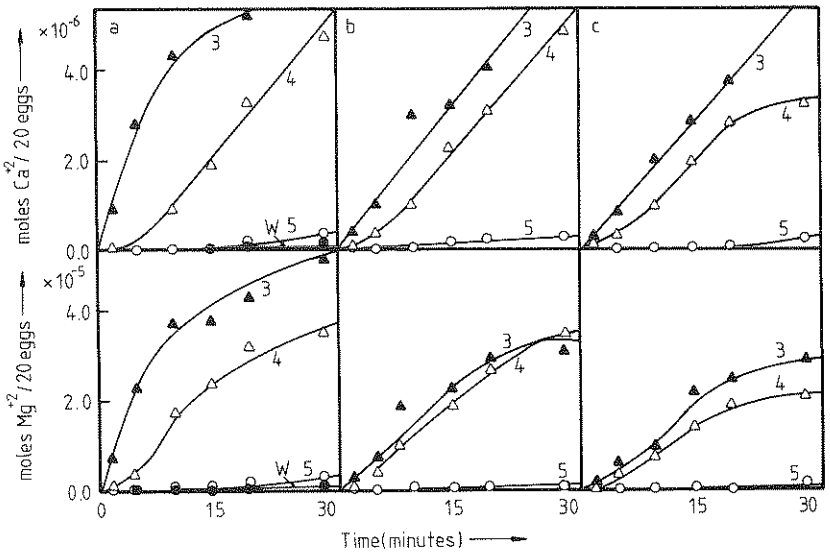

Fig. 1. Leakage of Ca^{2+} and Mg^{2+} from 20 Chinook salmon eggs as affected by varying acidity: (pH = 3.0, 4.0, 5.0 and in distilled water, W, 5.6–6.0) (a) sulfuric acid, (b) nitric acid, and (c) hydrochloric acid, volume 200 ml.

data were normalized to losses of divalent cations or "glycine equivalents" per 20 fish eggs or g dry weight of gill, and plotted as a function of time. The corresponding losses into distilled water of pH 5.6–6.0 were determined for comparison. Points are the average of two experiments.

RESULTS

Eyed Chinook salmon eggs

Figure 1 contains data on the losses of Ca^{2+} and Mg^{2+} from eyed Chinook salmon eggs induced by immersion in sulfuric, nitric and hydrochloric acid solutions of varying pH (5.0, 4.0 and 3.0). Experiments were also carried out in distilled water for comparison. The results show that decreasing pH enhances to losses of Ca^{2+} and Mg^{2+}. In several cases even at pH = 5 and at longer times losses of these divalent cations exceeded those in distilled water.

The losses of amines, measurable by the fluorescamine method from the fish eggs in sulfuric, nitric and hydrochloric acid solutions of varying pH (5.0–3.0) and distilled water were measured. The results show that within experimental error losses at pH's of 4.0 and 5.0 for sulfuric and hydrochloric acids, and pH's of 3.0, 4.0 and 5.0 for nitric acid to be the same as that in distilled water. Primary amine losses in sulfuric and hydrochloric acids at pH = 3.0

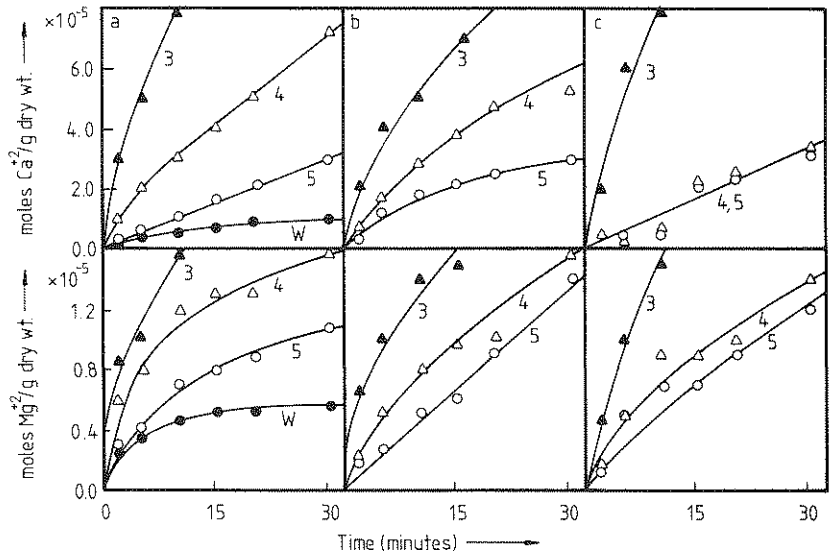

Fig. 2. Leakage of Ca^{2+} and Mg^{2+} from excised gills of *A. californiensis* as affected by varying acidity: (pH = 3.0, 4.0, 5.0 and in distilled water, W, 5.6–6.0) (a) sulfuric acid, (b) nitric acid, and (c) hydrochloric acid, volume 200 ml.

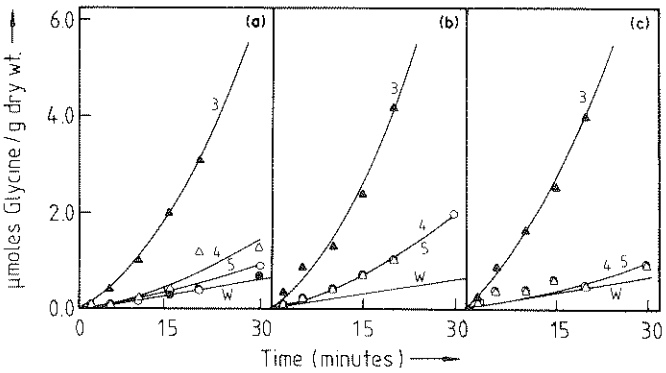

Fig. 3. Leakage of fluorescamine measured primary amines from excised gills of *A. californiensis* as affected by varying acidity: (pH = 3.0, 4.0, 5.0 and in distilled water, W, 5.6–6.0) (a) sulfuric acid, (b) nitric acid, and (c) hydrochloric acid, volume 200 ml.

are greater than that in distilled water. For comparison, the μmoles glycine/20 eggs is 0.0 at 30 min for distilled water, 0.5 ± 0.1 for sulfuric acid, and 0.3 ± 0.1 for hydrochloric acid.

Gills of Anodonta californiensis

Figure 2 contains data on Ca^{2+} and Mg^{2+} losses for excised gills of the freshwater, bivalve mollusc *A. californiensis* with sulfuric, nitric and hydrochloric acid solutions of varying pH (5.0, 4.0 and 3.0). Even at pH = 5 Ca^{2+} and Mg^{2+} losses exceed those in distilled water.

Figure 3 shows the effects of sulfuric, nitric and hydrochloric acid solutions of varying pH (5.0–3.0) on the loss of primary amines from excised gills compared to that in distilled water. Clearly increasing acidity from these acids causes increased primary amine losses. At pH = 4 and at longer times amine losses can exceed those in distilled water.

DISCUSSION

Several interesting conclusions evolve from this work which suggest how acid rain and the resulting acid freshwater systems begin on a molecular scale the disruption of organisms. In this work we have chosen to examine an encapsulated living organism, eyed Chinook salmon eggs, and the excised gill tissue from a ubiquitous, freshwater, bivalve mollusc, *A. californiensis*.

For both eggs of *O. tshawytscha* and gills of *A. californiensis* Ca^{2+} and Mg^{2+} losses increase with increasing hydrogen ion concentration, decreasing pH (Figs 1 and 2). Even at pH = 5 the losses of Ca^{2+} and Mg^{2+} can exceed those in distilled water. The effect at pH = 5 is greater for gills of *A. californiensis* than for eggs of *O. tshawytscha*. In another study (Swinehart *et al.*, 1984) we have shown that the losses of Ca^{2+} and Mg^{2+} are unaffected by the presence of Mg^{2+} and Ca^{2+}, respectively, and are the same as those into distilled water. Therefore, the losses of Ca^{2+} and Mg^{2+} even at pH = 5.0 in natural waters are very likely greater than losses into water of higher pH. The conclusions reached from these observations is that for both biological systems, eyed salmon eggs and excised gill tissue, water at the low acid boundary of acid rain, pH = 5.0, induces losses of Ca^{2+} and Mg^{2+} from membranes in excess of those observed under non-acid rain conditions. In other work, *in vivo* studies of the crayfish *Orconectes virilis* have shown that Ca^{2+} uptake and biological processes involving calcium such as the calcification of the exoskeleton are inhibited by acid at pH's near 5 (Malley, 1980) Primary amine losses from the gills of *A. californiensis* and eggs of *O. tshawytscha* are enhanced with increasing acidity. The gills of *A. californiensis* are more sensitive to acid than eggs of *O. tshawytscha.*

The general conclusions reached from this study are: (1) even at pH = 5 the losses of Ca^{2+} and Mg^{2+} from both biological systems exceed those found in waters of higher pH (non-acid waters) within minutes after exposure to such waters, and (2) increasing acidity increases short time primary amine loss from both biological systems.

Organisms in acid environments will have long term exposures to water in the pH range 4.5 and higher due to the buffering effect of the HCO_3^-–CO_2 equilibrium. Short term exposures to waters of pH approx. 4 are possible under conditions where the water flux is large and/or the HCO_3^-–CO_2 equilibrium is not attained (Harvey, 1979; Leivestad *et al.*, 1976). Considerations concerning the environmental impact of this study should be limited to the results at pH = 4.0 and 5.0.

From the point of view of an ecologist the most important conclusion of this study is that even at pH = 5, normally defined as near the high pH or low acid boundary of acid waters, there are immediate, observable, and large losses of divalent cations (*O. tshawytscha* and *A. californiensis*) and at pH = 4 primary amines (*A. californiensis*). Since divalent cations are thought to stabilize biological membranes (Watt and Pierce, 1978 a,b; Papahadjopoulos, 1978) the losses of Ca^{2+} and Mg^{2+} will compromise the integrity of the membranes. The loss of membrane integrity is seen in the increased efflux of primary amines.

Clearly acid waters produce other effects on organisms. For example, McDonald *et al.* (1980) have shown that when rainbow trout, *Salmo gairdneri*, are exposed to waters at pH = 4.3 and low Ca^{2+} content (0.3 mM) their plasma Na^+ and Cl^- levels decline and mortality increases compared to a control group and a group exposed to water having a high Ca^{2+}

content (~ 3 mM) and pH = 4.3. The conclusion of the above study is that ionoregulatory failure is the toxic mechanism at low pH. This study suggests that Ca^{2+} and Mg^{2+} losses from membrane induced by increased acid are at least partially responsible for this ionoregulatory failure.

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