Bull. Environm. Contam. Toxicol. 21,569-575 (1979)

The Effects of Cations on Sodium Fluxes in the Presence of Chlorine

Bernice M. Katz

Department of Oceanography, Florida Institute of Technology, Melbourne, Fla. 32901

Fish mortality to chlorine has been attributed to impairment of gill function (BASS et al. 1977, PENZES 1972) and to oxidation of hemoglobin resulting in inhibition of respiration (GROTHE and EATON 1975). Introduction of fish to a low concentration of chlorine (less then 1 ppm) causes an immediate and significant efflux of sodium and gain in weight reflecting an ionic and osmotic imbalance. In addition the toxicity of chlorine has been shown to be influenced differentially by cations prevelant in natural waters - sodium, potassium, calcium and magnesium (KATZ 1975). Because of this it is presumed that an influx of water due to an osmotic imbalance at the gill membrane is the direct cause of gill impairment and mode of toxic action under certain conditions. This study was undertaken to determine if these cations have a differential effect on sodium fluxes in the presence and absence of chlorine and if this relates to the protection and/or potentiation of toxicity to chlorine.

MATERIALS AND METHODS

Adult Gambusia affinis, collected from the stabilization ponds of the local sewage treatment plants, were the test fish chosen to compare with previous toxicity determinations (KATZ 1975). They were kept in the water that they had previously been acclimated in addition to acclimation in NaCl equivalent to 25% seawater and in distilled deionized (D.D.) water to test the effects of prior acclimation in various ionic strengths. Reagent grade chemicals were used for the cations. Differing concentrations of potassium, calcium and magnesium were prepared in D.D. water in the presence and absence of chlorine. Commercial bleach (Chlorox®) was used for preparation of the chlorinated solutions. Equal amounts of chlorine (0.32 ± 0.01 mg/L) were added to all chlorine media.

For the sodium efflux studies, fish were quickly weighed on a single pan balance and transferred to the test solutions. They were treated equivalently to insure that stresses caused by handling were alike in controls and test fish so that measured differences were due only to water content. Aliquots of 5 mL were taken

0007-4861/79/0021-0569 $01.40

before introduction of fish to be used as the blank and during 15, 30 and 60 minute intervals. Sodium ion was measured on a Jarrell Ash atomic absorption spectrophotometer in the emission mode. Sodium fluxes were compared to the mean control of freshwater acclimated fish placed in D.D. water. This consisted of 10 random batches of fish collected at different times in order to insure reproducibility of the method. The means and standard deviations of the control are compared with test runs. Combined chlorine was measured by the o-tolidine method as outlined in Standard Methods (AMERICAN PUBLIC HEALTH ASSOCIATION). Only acute short term effects were studied.

RESULTS AND DISCUSSION

Acute exposure of freshwater acclimated Gambusia to chlorine resulted in a marked net efflux of sodium in both rate and quantity, Figure 1. Calcium and magnesium reduced sodium effluxes in all concentrations tested. Only results where the fish remained alive during the course of the test run are reported. In Figure 1, calcium and magnesium equivalent to their concentrations in 25% seawater lowered sodium effluxes due to chlorine but did not reduce them to control levels. Calcium at the same concentrations reduced the efflux of sodium slightly below the control.

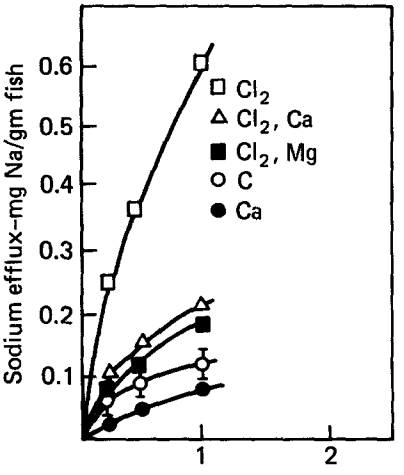

Fig. 1

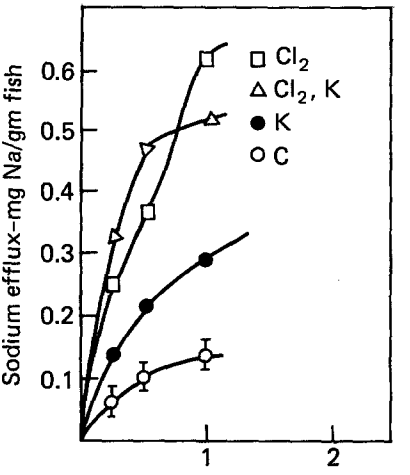

Fig. 2

Fig. 1. Sodium efflux of fish exposed to chlorine alone and with Ca and Mg; control (C) fish in D.D. water with and without Ca.

Fig. 2. Potassium with chlorine significantly increased sodium effluxes above control.

In contrast to calcium and magnesium, potassium in all concentrations tested, caused a substantial net increase in sodium efflux. In Figure 2 are the results of 25% seawater equivalent potassium with and without chlorine. The addition of chlorine to a potassium solution produced a marked increase in sodium efflux over potassium. This efflux surpassed chlorine in D.D. water in rate but during the course of the experiment was reduced to less than chlorine. Potassium equivalent to 0.125% NaCl actually had a higher efflux alone than with chlorine.

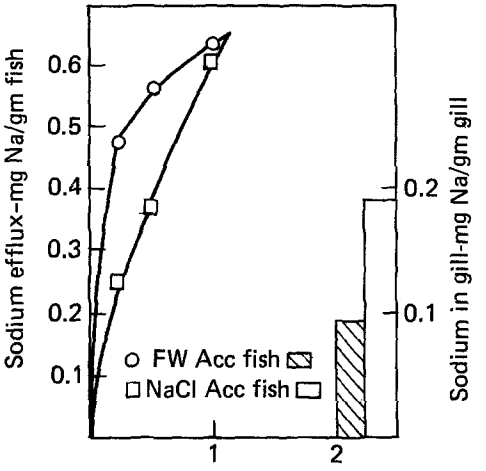

Fig. 3

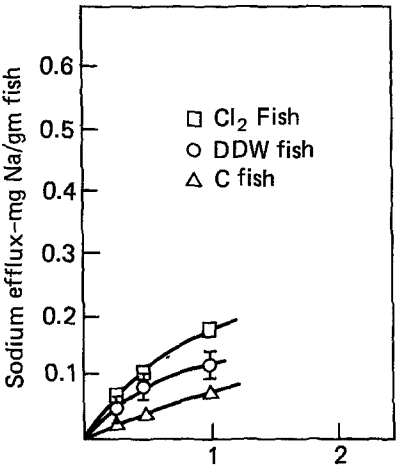

Fig. 4

Fig. 3. Comparison of sodium effluxes in chlorine, and Na in gills from fish acclimated in fresh water and in enriched salt water.

Fig. 4. Lowered effluxes of sodium from fish acclimated in D.D. water and fish surviving chlorination.

Acclimation in varying concentrations of saline water was shown to have a pronounced effect on chlorine induced toxicity. Fish acclimated in 25% seawater showed a marked resistance to chlorine when placed in tapwater in contrast to high mortalities when acclimated in freshwater (KATZ 1975). In Figure 3 the efflux of sodium of fish acclimated to NaCl equivalent to 25% seawater prior to transfer in chlorinated D.D. water is similar to that of fish acclimated in freshwater. Analyses for sodium in the gill of the acclimated fish prior to placing in chlorine showed twice as much sodium in the saltwater acclimated fish. Fish acclimated in D.D. water and surviving chlorine intoxicated fish when placed in a chlorine media produced sodium effluxes less than fish acclimated in waters with high sodium content (Figure 4).

Acute sodium effluxes appear to reflect overall toxicity effects due to chlorine including the influence of cations. An attempt was made to relate this efflux to mechanical damage of the gill epithelium (PENZES 1972) by application of the van't Hoff equation: $P = RTC$ where P, the osmotic pressure is directly proportional to R, the universal gas constant and T the absolute temperature which are constants, and C, here taken to be the difference in concentration of sodium inside and outside the membrane. Although the exact volume of water at the gill membrane could not be determined, 0.5 mg and more of total expelled sodium, converted to moles, divided by a small volume and inserted in the equation, could account for a large osmotic gradient to produce a sufficient hydrostatic pressure to account for considerable gill damage. Weight gain of fish in the presence of chlorine (Figure 5) is then interpreted as an influx of water. Decreases in toxicity of saline acclimated fish could be the result of a reserve of sodium in the blood so that loss of sodium is not as detrimental as in the freshwater acclimated fish. DAVSON (1970) reported the classic work of Shaw and Bryan where the rate of sodium uptake varied directly with external concentrations and inversely with internal concentration. It appears that the concentration of sodium present in the blood would also have a marked influence on the detrimental effect

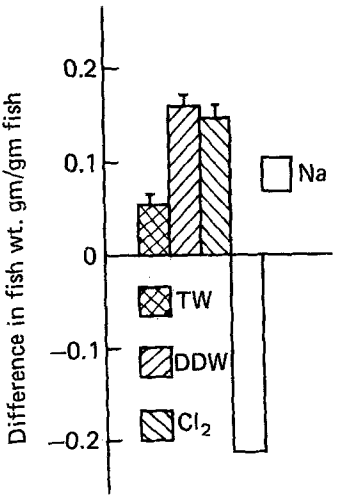

Fig. 5

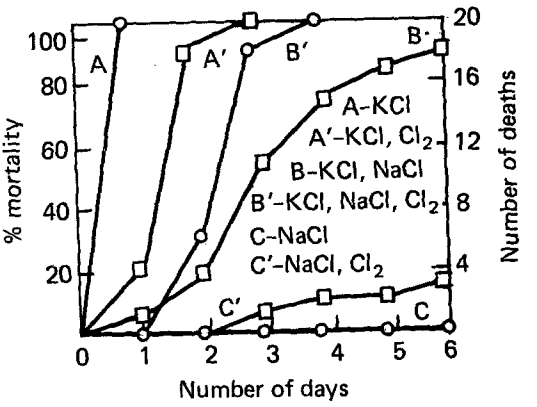

Fig. 6

Fig. 5. Comparison of weight changes in fish after 5 hrs. in tap water, distilled water, chlorinated tap water and NaCl.

Fig. 6. Cumulative toxicities of potassium, sodium and potassium with sodium prior to and after addition of 0.5 ppm chlorine and total cation concentrations equivalent to 0.125% NaCl (from KATZ 1975).

of chlorine in the same manner as low external concentrations of sodium. This is also borne out in the low effluxes of sodium of fish kept in distilled and chlorinated waters (Figure 4). Although not determined, this is believed to be the result of an internal depletion of sodium.

Cationic influence concurs with diminished and enhanced sodium effluxes. Although calcium lessened sodium efflux of chlorine in a short time, in a 6-day period the ion did not reduce toxicity of chlorine and actually nullified the protective action of sodium ion (KATZ 1975).

Toxicities due to potassium equivalent to 0.125% NaCl in the presence and absence of chlorine and NaCl are summarized in Figure 6. An efflux of sodium in the presence of a high concentration of potassium could possibly be offset by chlorine reducing membrane permeability to potassium which in high concentrations is more detrimental than chlorine. EVANS (1975a) reported that potassium lowers influx of sodium and (1975b) the addition of calcium to the external media reduces sodium efflux. This concurs with the above results. The presence of chlorine greatly modifies the actions of these cations.

At this point it is interesting to compare these results with heavy metals. For example it has been reported that hardness and salinity protect fish from zinc and cadmium (SINLEY et al. 1974, EISLER 1971). In a series of tests it was shown that silver, copper, zinc and cadmium cause a marked efflux of sodium and calcium equivalent to its concentration in 25% seawater reduces sodium efflux due to these metals to control levels (KATZ 1976). The results of 5 ppm cadmium is shown in Figure 7. COURTOIS and MEYERHOFF (1975) reported that the breakdown in water balance at the Na^+-H^+ exchange site on the gill could be the mode and site of action of copper ion. This was based on weight increase and serum Na^+ decrease. Weight increase and sodium efflux in the presence of chlorine appear to parallel the results of COURTOIS and MEYERHOFF (1975). BASS et al. (1977) reported that their physiological studies with chlorine are similar to other authors' results with varying compounds, one of which was SKIDMORE'S (1970) findings with zinc, and these reflect an impairment of gill function. Although the chemistry of heavy metals and chlorine differ, the mode of action appear to be similar except in the protection incurred by calcium to metals. Exactly what occurs at the membranal level to cause a breakdown in permeability barriers by these substances can only be speculative at present. It can be presumed that even if sufficient sodium ion exists to protect the gill from mechanical damage, greater amounts, 1.5 ppm as chloramine (GROTHE and EATON 1975) could cause toxicity by another mode which as reported is oxidation of hemoprotein.

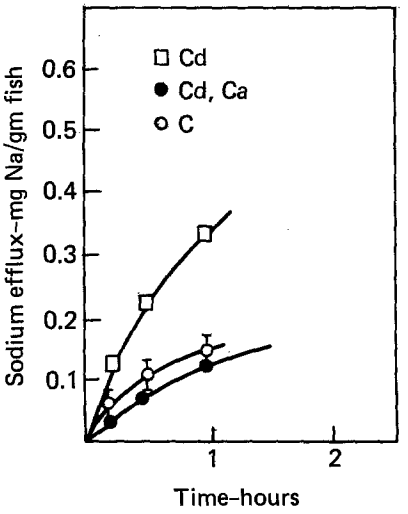

Fig. 7. Effect of 5 ppm Cd alone and with Ca on sodium effluxes. Ca equivalent to its concentration in 25% seawater nullifies the effect of Cd.

Fig. 7

At the environmental level the cationic composition of natural waters could have a great bearing on the effect of a chlorine discharge. At present it would be difficult to apply these preliminary results to actual conditions, as this study was aimed only to establish that there is a pronounced interplay of cations with chlorine in modifying sodium fluxes and toxicities. The chronic nature of these manifestations and effects of the multitude of combinations of these cations has yet to be resolved. In addition in natural waters many other types of compounds can be present such as nitrogenous substances which were also found to alter the toxicity of chlorine (KATZ 1977). However the protection of sodium, nullification by calcium and in some unusual cases protection from potassium by chlorine, confirm the complexity of chlorine-induced lethality and the care that must be excercised in interpreting toxicities in waters of varying composition.

ACKNOWLEDGEMENT

Critical review of this manuscript by Dr. J.A. Lasater is appreciated.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION: Standard methods for examination of water, sewage, and industrial wastes, 13th ed. New York 1971.

BASS, M.L., C.R. BERRY, Jr. and A.G. HEATH: Water Res. 11, 731 (1977).

COURTOIS, L.A. and R.D. MEYERHOFF: Bull. Environ. Contam. Toxicol. 14, 221 (1975).

DAVSON. H.: A Textbook of General Physiology Vol. I. Baltimore, The Williams and Wilkins Co. 1970 Pg. 988.

EISLER, R.J.: Fish Res. Bd Can. 28, 1225 (1971).

EVANS, D.H.: J. Comp. Physiol. 96, 111 (1975a).

EVANS, D.H.: Comp. Biochem. Physiol. 51A, 491 (1975b).

GROTHE, D.R. and J.W. EATON: Trans. Amer. Fish. Soc. 104, 800 (1975).

KATZ, B.M.: Physiological Actions of Low Concentrations of Chlorine to the Mosquito Fish Gambusia affinis. Master's thesis, Florida Institute of Technology, Melbourne, Florida 1975.

KATZ, B.M.: Mechanism of Entry of Metals in the Fish Gill. Presented at the 49th Annual Conference of the Water Poll. Con. Fed., Minn., Minn. Oct. 3, 1976.

KATZ, B.M.: J. Water Poll. Contr. Fed. 49, 1627 (1977).

PENZES, B.: Biological Abstracts 53, #34643 (1972).

SINLEY, F.R., J.P. GOETTL, Jr. and P.H. DAVIES: Bull. Environ. Contam. Toxicol. 12, 193 (1974).

SKIDMORE, J.F.: J. Exp. Biol. 52, 481 (1970).