REVISTA ESPAÑOLA DE FISIOLOGIA, 38, 339-344. 1982

# Oxygen Uptake by Dogfish Gill Tissue after Several *in vitro* Zinc Treatments

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(Received on March 15, 1982)

L. TORT, R. FLOS and J. BALASCH. *Oxygen Uptake by Dogfish Gill Tissue after Several in vitro Zinc Treatments.* Rev. esp. Fisiol., 38, 339-344. 1982.

Oxygen consumption by dogfish gill tissue is measured after several *in vitro* zinc treatments ranging from low contamination (0.2 ppm) to the 50 % lethal concentration at 24 hours (180 ppm). Zinc inhibits respiration from 15 ppm and the inhibition increases along with greater zinc concentrations from 15 to 60 ppm. Thereafter the respiration level becomes stabilized with no further inhibition increases.

A similar pattern is described with homogenates but inhibition is stronger and faster, while respiration does not stabilize.

The results are discussed in relation to the possible role of the membrane and the toxicological effects caused by zinc.

Heavy metals, above physiological range, may act as toxic chemical substances (4, 5). They act ultimately at cellular and molecular level disturbing cellular functions by physico-chemical reactions that impair biochemical mechanisms. Metals affect respiration of organisms at different levels: changes in ventilatory rhythms (16, 27), changes in oxygen uptake of gill tissue after metal treatments with copper (3, 20, 25), chromium (15), silver (28), cadmium and mercury (13) and zinc (3, 29). In gills, at cellular and molecular levels several effects have been described: decrease of aminoacid uptake, and protein synthesis, diminution of ATP content (32), inhibition of NADPH (7, 22), mitochondrial respiration (33), mitochondrial electron transport (21), and lactate oxidation (2). In addition, structural changes have been reported in gills due to heavy metals (9, 11).

The aim of this paper is to study the effect of zinc on the tissue respiration of dogfish gills *in vitro*. Oxygen uptake (as an indicator of cellular metabolism) is measured in intact tissue as well as in homogenates after zinc treatments to see whether the membrane plays a role as a site of biochemical interactions when zinc is present, and to know the effect of zinc on tissue respiration when cytoplasmic systems are not protected by the membrane diffusion barrier.

## Materials and Methods

Dogfish *(Scyliorhinus canicula,* L) of both sexes, body weight between 100 and 350 gr. were used. After collection, fish were kept for at least a week in an open circulation tank (natural seawater, pH = 7.7-7.8, salinity = 35.8 %-37.2 %, temperature = 14-16° C).

The study was carried out in two sets of experiments using either isolated gill tissue or homogenized tissue. The effect of several concentrations of zinc on oxygen consumption was tested. Concentrations of Zn as $ZnSO_4$ were 0.2 ppm, 1 ppm, 5 ppm, 15 ppm, 30 ppm, 60 ppm, 120 pp and 180 ppm, i.e. a range from low contamination to the 50 % lethal concentration at 24 hours (180 pm) (12). Oxygen consumption was measured by the direct method of Warburg (1). Temperature was maintained at 20° C.

For isolated tissue, after killing fish by decapitation, ten portions of gill tissue (60-80 mg) were excised from each fish, weighed, and placed in Warburg flasks containing artificial seawater (8), while desiccation during handling was avoided. Readings were performed every 60 minutes. For each fish five samples were maintained as controls and five samples were treated with zinc: zinc sulphate solutions were added to flasks after 1 hour of respiration. Readings were made for at least five hours after zinc addition, and, oxygen consumption given in $\mu$l $O_2$/mg h. Each fish was tested for one concentration and 9-14 fishes were used for each concentration.

For homogenized tissue, after decapitation and dissection, gill tissue was homogenized with artificial sea water in the proportion of 60 mg of tissue per 2.5 ml of water. Ten aliquots corresponding each to 60 mg of gill tissue were placed in Warburg flasks. Readings were performed every 15 minutes. For each fish five samples were maintained as controls and five samples were treated by adding zinc sulphate from the start of the experiments. The performance of homogenized tissues falls very quickly in comparison to intact tissues, therefore readings were done during one hour and oxygen consumption was also given in $\mu$l $O_2$/mg h. Each fish was tested for one concentration and 9-14 fish were used for each concentration.

Results were analyzed after the Student «t» tests (independent samples and paired tests) and correlation tests. In all cases 0.05 was taken as the level for which significance was accepted. In order to compare respiration inhibition rates in the different treatments, a percent index of respiration in zinc treated tissue in relation to their own controls was calculated. The following formula was used for each fish:

$$R = 100 - \frac{QO_2c - QO_2t}{QO_2c} \times 100$$

$R$ = Percentage of respiration.

$QO_2c$ = Oxygen consumption mean of the five control samples.

$QO_2t$ = Oxygen consumption mean of the five treated samples.

## Results

Several parameters were previously studied in order to determine any possible correlation between oxygen consumption and sex, body weight or position of branchial arch. Positive correlation was found between body weight and oxygen consumption but no significant correlation existed between sex or branchial arch position and oxygen consumption. Therefore in the experiments each fish was used as its own control.

Figure 1 shows an example of the inhibition of intact gill tissue respiration caused by zinc during the treatment (15 ppm in this example). Oxygen consumption follows a linear pattern and cells

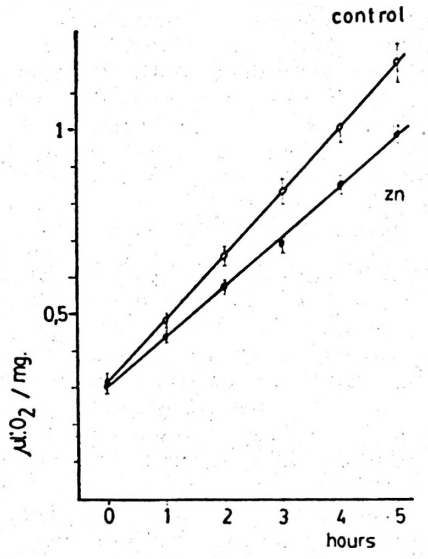


Fig. 1. *Oxygen consumption of control and treated (15 ppm Zn) samples of one fish during 5 hours.*
Mean ± standard error of the mean.

can respirate in the Warburg flask for at least 8 hours after gill removal without decay, even in the presence of Zn.

Figure 2 shows the respiration curves in both intact and homogenized tissues at the different zinc concentrations from 0.2 ppm to 180 ppm. In intact tissue

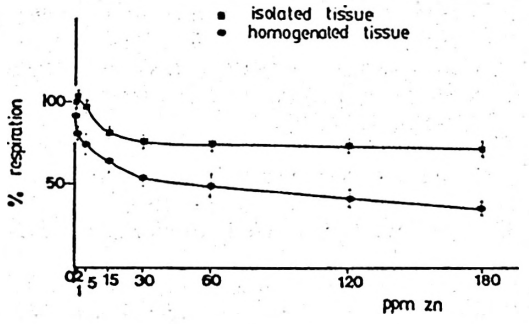


Fig. 2. *Oxygen consumption percentage of isolated and homogenized tissue following increasing concentrations of zinc.*
Mean ± standard error of the mean.

below 5 ppm of zinc, the effect of the metal on tissue respiration is not significant. Between 5 and 30 ppm a strong decrease is shown. After 30 ppm the pattern of the curve shows a plateau, at least in the range of concentrations tested, and the inhibition becomes stabilized.

In homogenized tissue respiration, inhibition is greater than in intact tissue. Below 1 ppm the effect of zinc on oxygen consumption is not significant. Between 1 and 30 ppm of zinc, a greater decrease in respiration is observed. Above 30 ppm, inhibition slowly increases as zinc concentration increases and does not reach a plateau in the range of concentrations tested. So, when the curves in both intact and homogenized tissue are compared, a similar pattern is shown up to 30 ppm with a stronger and quicker inhibition for homogenized tissue. Above 30 ppm a different inhibition pattern has been recorded, in which respiration reaches a plateau in intact, but not in homogenized tissue.

## Discussion

There is no general agreement on the relationship between oxygen consumption and body weight. Positive (10), negative (1) and non significant correlations (14) have been found. This disagreement could be due to differences between species and also between tissues. Differences in oxygen consumption related to sex have been reported in crab and salmon (1, 19) by measuring whole animal oxygen consumption per weight unit, but no clear differences have been observed in relation to sex and gill tissue oxygen consumption (14).

Correlations between position of branchial arch and tissue oxygen consumption in decapoda (14) as well as correlation between position of branchial arch and metal accumulation in dogfish gill tissue have also been established (11, 14) but

no differences have been found in the present work between position of branchial arch and gill tissue oxygen consumption.

In relation to metal effects on oxygen consumption, it must be said that zinc causes a different pattern of inhibition on homogenized and intact tissue, which is greater on homogenized tissue. Other authors have found no effect on the respiration of homogenized mussel gill tissue after the addition of zinc sodium citrate or copper sodium citrate (3). These results may be explained by the presence of citrates in the medium, which might exert an opposite effect on the diminution of respiration caused by the metal. When the metal is not present, citrate does not increase tissue respiration. Unpublished research from our laboratory studying the effects of zinc, glucose or citrate, and zinc plus glucose or citrate on gill tissue respiration would agree with this idea.

Having compared the results on intact tissue and on homogenates, an important role of the membrane in protecting the enzymatic activity of respiratory enzymes against the toxicity of heavy metals might be suggested. Zinc action can be produced at different levels: ROTHSTEIN (24) suggests that the membrane is the most important site of action for heavy metals which could act on the membrane through several mechanisms: Altering bioelectric potentials, changing permeability, affecting transport processes, or affecting enzymatic systems (17). Alterations of specific membrane resistance by zinc (23) and of membrane water permeability by lead (6) have been described and KATZ (18) suggests that heavy metals cause alterations in gill membrane permeability. Metal ions could interfere with transport function or bring structural alterations (30) through their attraction to negatively charged cell membranes. Some authors (26) have also found inhibition of fish gill $Ca^{++}$ ATPase caused by zinc and other metals.

*Acknowledgements*

Authors are indebted to Dr. P. Arté from the «Instituto de Investigaciones Pesqueras of Barcelona» for his technical assistance and help.

## Resumen

Se estudia el consumo de oxígeno del tejido branquial del pez lija (*Scyliorhinus canicula* L.) después de diferentes tratamientos *in vitro* con zinc, desde concentraciones de baja contaminación (0,2 ppm) hasta la concentración letal a las 24 horas (180 ppm). Los resultados muestran una inhibición de la respiración debida al zinc, a partir de 15 ppm, que es creciente cuando se aumenta la concentración hasta 60 ppm. A partir de 60 ppm la inhibición no aumenta.

Con branquia homogenizada se observa un patrón parecido de inhibición que, aunque más pronunciado y rápido, no llega a estabilizarse.

Los resultados se discuten en relación al posible papel de la membrana y a los efectos tóxicos del zinc.

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