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Gill Damage as a Determinant of Residual Oxygen Concentration in the Sealed Jar Test

Heikki Tuurala, Mikko Nikinmaa, and Antti Soivio

Department of Zoology, Division of Physiology, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki 10, Finland

The residual oxygen bioassay or sealed jar test is used as a rapid, simple means for measuring effects of toxicants on the ability of fish to withstand oxygen depletion. Healthy fish are placed in a sealed jar, which contains a known concentration of toxicant, and allowed to consume oxygen until death occurs. The residual oxygen concentration is then measured and an elevation in the oxygen concentration indicates a harmful effect of the toxicant. The threshold-effect concentrations are approximately the same as those obtained in 96-h LC_{50} tests (McLeay 1976; Gordon and McLeay 1977; Vigers and Maynard 1977; Giles and Klaprat 1979).

The sealed jar test thus supposes that the effect of toxicant on the respiration is very rapid, almost instantaneous, taking place in healthy fish during the test. To test if this assumption is valid, we have carried out a series of experiments with zinc, which causes extensive gill damage (Skidmore and Tovell 1972), first by pre-exposing fish to zinc and measuring residual oxygen concentration after sealed jar test, secondly by putting healthy fish in sealed jars with known concentration of zinc and measuring residual oxygen concentration, and comparing both the above results with residual oxygen levels of healthy fish in clean water.

Additionally we have investigated the role of gill damage in determining the residual oxygen concentration by ligating gill arches, thus limiting effective gill surface area, and carrying out sealed jar tests in clean water. In order to see if tissue oxygen consumption may influence the residual oxygen concentration, the tests have been carried out at different temperatures.

MATERIALS AND METHODS

The experiments were carried out at Laukaa Fish Culture Research Station in Central Finland in August 1979 - June 1980. The water was obtained from a nearby lake (Peurunkajärvi) and the water quality during the experiments was the following: pH 6.3 - 7.1, PCO_2 < 100 Pa, oxygen saturation > 80 %, specific conductivity at 20 °C 36 - 40 µS/cm and total hardness 0.16 mmol $CaCO_3$/L. The fish used were 0 - 1-year-old rainbow trouts (*Salmo gairdneri*) weighing from

8 to 160 g from the local stock or from a commercial fish farm (Savon Taimen Oy, Rautalampi).

Before the experiments the fish were kept indoors at least 2 weeks in 300 L (1 m^2) glass fiber tanks at naturally prevailing temperatures (2 - 18 °C). The fish were fed daily with pelleted trout food until the experiments begun.

Twentyfour hours before the sealed jar tests the fish were lightly anaesthetized with MS-222 (Sandoz) (1 : 10 000) and agraffs (Martin, Art. 5-960) were placed and tightened with pliers on the I, I and II or II and III gill arches on both sides in order to stop the blood flow in respective arches. With some fish the obstruction of blood flow was checked by injecting Evans blue solution in *bulbus arteriosus* and following its distribution in the gills. Almost no Evans blue was detected in the secondary lamellae and filaments of the ligated gill arches. The control fish of these groups were also anaesthetized at the same time.

Zinc sulphate ($ZnSO_4$) was used to produce the chemical gill damages. Two different concentrations were used (1.9 and 2.5 mg/L as Zn). The fish were exposed to zinc for 24 hours before the residual oxygen measurements in a static system. One group was kept in clean water and the sealed jar test was carried out in water containing 2.5 mg/L zinc.

A group of fish was acclimated for 24 hours from 2 °C to 10 °C and the tests were made in clean water at 10 °C.

In some groups the thickness of the secondary lamellar epithelium was measured as mean harmonic distance from the outer surface of the secondary lamellar epithelium to the margin of the blood space walls in the secondary lamellae (Soivio and Tuurala 1981). The measurements were carried out from 1 µm thick plastic sections made from the gills of zinc exposed and control fish.

Test containers for sealed jar tests were 2 L wide mouth glass bottles in August 1979 (gill arch ligations at 17 °C) and 1 L wide mouth polythene bottles for all other tests in 1980. The bottles were filled with clean air-saturated water (except in the group where zinc was added in the bottles) and the fish were put individually in the bottles with minimum disturbance. The vessels were sealed and placed in the holding tanks. The time of death of each fish was recorded (the time of death was judged from the ceasing of opercular movements), the vials were weighed, dissolved oxygen concentration measured with Winkler method. The fish were weighed after the water sample for oxygen measurements was taken.

RESULTS AND DISCUSSION

The tests with zinc (Table 1) clearly show that at the low temperature (+2 °C) used the original sealed jar test does not give any indication of the harmful effects of toxicant. Zinc causes extensive gill damage by detaching the secondary lamellar epithelium and

fusing the adjacent secondary lamellae together, thus decreasing the effective gill surface area and increasing the diffusion distance from water to blood (Tuurala and Soivio 1982; Tuurala 1983). This was observed in this study, as the mean harmonic distance from water to blood increased from 2.8±0.2 µm in control fish to 7.3±0.7 µm in the group exposed to 2.5 mg/L zinc for 24 hours ($n = 10$, $p < 0.001$, Student's t-test). The time course of these changes is unknown. The present results show that the time taken (about 2 h) for the fish to die in the bottles we used did not cause any damage, as seen from the residual oxygen concentration. When the fish were pre-exposed to zinc for 24 h a clear increase of residual oxygen concentration was observed. This is a major drawback of the system and may preclude the use of residual oxygen test at least at low temperatures. The 'reverse' sealed jar test i.e. putting exposed fish in clean water, on the other hand, gave a clear change in residual oxygen concentration.

Table 1. Zinc-induced gill damages.

A. Tests at 10 °C Group	RO_2 mg/L	weight g	n
1. Control fish	1.64±0.08	57.2±1.4	10
p 1 - 2	*	NS	
2. Zn 1.9 mg/L	2.04±0.13	56.0±3.1	10
B. Tests at 2 °C			
1. Control fish	1.31±0.09	9.8±1.2	9
p 1 - 2	***	NS	
2. Zn 2.5 mg/L	2.99±0.23	8.9±1.1	7
p 1 - 3	*	NS	
3. From 0 to 2.5 mg/L Zn	1.04±0.07	10.6±1.3	10

Residual oxygen concentration (RO_2), weight of experimental fish and number of observations. Mean ± SEM are presented for each group.
Significance levels refer to Student's t-test. NS = no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Gill damage seems to be the major determinant of residual oxygen concentration in the sealed jar test. No change in residual oxygen concentration was observed when fish were placed in sealed jars and allowed to use up O_2 from clean water at different temperatures (Table 2).

In contrast, we were able to obtain clear increase in residual oxygen concentration by decreasing effective gill surface area via ligation of gill arches (Table 3). Thus, the different effects of toxicants in the sealed jar tests at different temperatures (cf. Gordon and McLeay 1977) may rather reflect the different time course of gill damage at different temperatures.

Table 2. Effect of 24 h acclimation to higher temperature on residual oxygen concentration.

Group	RO_2 mg/L	weight g	n
1. Control fish	1.34±0.04	12.6±1.3	10
p 1 - 2	NS	NS	
2. From 2 to 10 °C	1.30±0.07	12.9±1.3	9

For explanations, see Table 1.

Table 3. Effects of ligation of gill arches.

Group	RO_2 mg/L	weight g	n
A. Ligations at 17 °C			
1. Control fish	1.18±0.06	114.8±10.7	11
p 1 - 2	NS	NS	
2. I arches ligated	1.44±0.12	101.8±6.9	10
p 1 - 3	***	NS	
3. I&II arches ligated	1.80±0.06	144.0±12.8	8
p 2 - 3	***	*	
B. Ligations at 8 °C			
1. Control fish	1.48±0.07	61.8±2.8	10
p 1 - 2	**	NS	
2. I&II arches ligated	1.88±0.10	58.7±3.3	10
p 1 - 3	**	NS	
3. II&III arches ligated	1.95±0.12	64.6±2.2	10
p 2 - 3	NS	NS	

For explanations, see Table 1.

On the basis of the above data we suggest that residual oxygen test gives mainly an indication of the effect of toxicants on gill structure, and that the accuracy of the test is dependent on the speed of changes in the effective gill area. We have obtained the most reproducible results by using 'reverse' sealed jar test in which gill damage is induced by short pre-exposure to toxicant, and thereafter the fish were subjected to the test in clean water.

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