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Effects of PCB on the Adrenergic Response in Perfused Gills and on Levels of Muscle Glycogen in Rainbow Trout (*Salmo gairdneri* Rich.)

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Polychlorinated biphenyls (PCB) are a heterogeneous class of industrial chemicals which have been intensively investigated concerning their role as environmental contaminants.

Alterations in behaviour have been observed in PCB exposed fish (BENGTSSON 1980, FINGERMAN & RUSSEL 1980, JOHANSSON et al. 1972, OLOFSSON & LINDAHL 1979). The response to external disturbance seems to be less evident in the PCB exposed fish than in unexposed ones (BENGTSSON 1980, HOLDEN 1965, JOHANSSON et al. 1972). A similar tranquillizing effect has also been observed in mice (MATTSSON et al. 1981), in Herringgulls (FOX et al. 1978) and in Coturnix quail chicks (KREITZER & HEINZ 1974) exposed to PCB. Furthermore, the levels of muscle glycogen are reported to be higher in fish exposed to PCB (FÖRLIN et al. 1979, JOHANSSON et al. 1972).

The question arose if these PCB related effects on fish were the result of an interaction between PCB and the "stress system".

Response to stress in fish is either mediated by neurons direct to the target organ or by catecholamines released to the blood circulation. The gills are sensitive to circulating adrenaline (WAHLQVIST 1982), increasing the oxygen uptake. Adrenaline also participates in mobilization of muscle glycogen.

A large number of studies on perfused gill preparations have demonstrated that catecholamines cause branchial vasodilatation by stimulating β-receptors in the gill vasculature (BERGMAN et al. 1974, PAYAN & GIRARD 1976, WOOD 1974, 1975). A small α-receptor mediated vasoconstriction in time preceeding the dilation has also been reported (BERGMAN et al. 1974, PAYAN & GIRARD 1976, WOOD 1975). Recent observations indicate that these alterations in blood flow through the gills increase the oxygen transfer to the blood (PETTERSSON 1982, PEYRAUD-WAITZENEGGER 1979, PÄRT et al. 1982 a). The purpose of this study was to find out whether PCB influences the adrenaline response in gills and/or glycogen storage in muscle.

MATERIALS AND METHODS

Perfused gill preparations from PCB exposed Rainbow trout were used. The advantage of this method is that controlled quantities of adrenaline can be administrated to the perfusion medium, replacing the blood and the response can be measured. The glycogen content in

white muscle was measured.

Rainbow trout (salmo gairdneri) were obtained in January 1981 from a local dealer (water temp. 0.5-1.0°C). The fish (130-170 g) were acclimatized for 30 days to the laboratory conditions in 1000 l holding aquaria supplied with Uppsala tap water (temp. 8-10°C) under imitated natural light-dark conditions. They were fed pelleted trout food (Astra-Ewos Trout food extra) daily. The fish were starved during the experimental period.

The experimental groups of twenty-five fish of both sexes were randomly selected and placed in two 300 L glas aquaria with circulating tap water and natural light-dark conditions. PCB (Aroclor 1254, batch 947940, Bayer AG, FRG) was dissolved in acetone and mixed with pelleted trout food. After evaporation of the acetone the food pellets were enclosed in gelatin capsules (size 3 Park, Davis & Company, GrB) in order to facilitate force feeding per os. Each fish was fed a capsule every second day, during a six day period, and received a total amount of. 0.173 g PCB. The control group was treated in an identical way but the PCB was omitted.

The perfusion experiment was carried out during a period of two to four weeks after the last capsule feeding. The isolated head was prepared principally according to the method of PAYAN & MATTY (1975). A detailed description of the preparation method and the perfusion system is given by Pärt et al. (1982a). The perfusion was carried out by a pulsative pump at a perfusion flow of 2.0 ml per 100 g fish and at a stroke frequency of 30-50 beats per min. Phosphate buffered saline in accordance to COLIN et al. (1979) was used as perfusion medium. The pH was adjusted to 7.40-7.45. The osmolarity was altered from 342 to 302 mOsm per kg by lowering the NaCl content. The plasma osmolarity of the fish used was 300-305 mOsm per kg (PÄRT unpublished data). Tap water (pH 8.0-8.2) was used as external medium and was continually aerated. The temperature of the experimental system was maintained at 11 $\pm$ 0.10°C. The sodium concentration of the external medium was measured every ten minutes throughout the experiment and analyzed with a flame photometer (Eppendorph, FRG). The pressure in the ventral aorta was measured with a pressure transducer (P23 Db, Stratham Gould Instr. Inc. GrB.) connected to a potentiometric recorder (Radiometer Servograph, Denmark). The oxygen tensions in the saline afferent and efferent to the gill and in the external medium were measured with polarimetric electrodes. The gill resistance (Rg) and the oxygen transfer factor (To_2) were calculated according to PÄRT et al. (1982a).

Three muscle samples of one gram each were taken by freeze clamping (BÖRJESON & FELLENIUS 1976) from white muscle in front of the back fin immediately after the head had been removed for perfusion. The samples were stored in -80°C and later treated as described by van HANDEL (1965) for glycogen analysis. The glycogen content was analyzed after hydrolysis with the glucose oxidase method (Glox Kabi-Vitrum, Sweden).
The PCB content in muscle, liver and gills was determined with gas-chromatography (JENSEN et al. 1979) at the National Swedish Environ-

ment Protection Board Laboratory, Wallenberg Laboratory, Stockholm, Sweden. Changes in Rg and To_2 are presented as per cent difference from the control value which is set at 100%. Statistical analysis of changes in Rg, To_2 and glycogen content between the control and PCB group has been carried out with independent samples (Wilcoxon rank-sum test). Significance level is set at P = 0.05 (two-tailed). Values are presented as means ± S.D.

RESULTS AND DISCUSSION

Contrary to expectation the response to adrenaline in both experimental groups consisted of a major α-adrenergic vasoconstriction followed by a small β-dilatation. In earlier observations a dominating β-adrenergic dilatation was reported (e.g. WAHLQVIST 1982). These new unorthodox results are discussed in a separate paper (PÄRT et al. 1982 b). In the present context the important finding was that there were no differences in adrenergic response in the gill vascular bed between PCB-exposed fish and the controls (Table 1).

TABLE 1. Changes in gill resistance after addition of adrenaline and/or phentolamine

	Gill resistance (Rg) %				Na^+-balance
Type of exp.	only saline	1 μM AD "peak"	1 μM AD "stabil"	10 μM phe no AD	10 μM phe 1 μM AD	$\mu mol \cdot h^{-1} \cdot 100\,g\ fish^{-1}$
Control n = 11	100	135±13 (ns)	121±11 (ns)	97±5 (ns)	89±4 (ns)	+6.7±9.9 (ns)
PCB n = 15	100	128±13	122±11	96±9	80±7	+6.1±3.3
Time	0-30	-	-45	-75	-90	(min)

Per cent changes in gill resistance (Rg) after addition of adrenaline (AD) and/or the α-adrenergic antagonist phentolamine (phe) to the perfusion saline. Drug concentrations refer to final concentrations in saline. $P < 0.05$ (Wilcoxon rank sum test, two tailed). The values are presented as means ± S.D. n = number of observations. A positive value of the Na^+-balance means a net uptake from the external medium.

In the presence of phentolamine, an α-adrenergic antagonist, adrenaline decreased gill vascular resistance significantly in both groups (Table 1). If PCB had impaired the sensitivity to adrenaline in the gill vascular bed the response to adrenaline should have been smaller in the PCB exposed group than in the control group.

Adrenergic responses increase the active gill area, which leads to a higher oxygen uptake (PETTERSSON 1982). In our experiments the

oxygen transfer factor (To_2) decreases about 10% in both experimental groups during the first 30 minutes. Addition of adrenalin leads to an increase of To_2 to the starting level (Table 2). Consequently there is no difference between the control and PCB exposed group with regard to To_2 mediated by adrenaline.

TABLE 2. Oxygen transfer factor (To_2) in %

Type exp.	no drugs t=10 min	no drugs t=30 min	1 µM AD t=45 min
Control n=11	100	87±13 (ns)	102±11 (ns)
PCB n=15	100	91±6	101±17

Per cent changes of O_2 in saline from dorsal aorta before and after addition of adrenaline. Drug concentrations refer to final concentration in the saline. Po_2 100% = 100 ± 12 mmHg. $P < 0.05$ (Wilcoxon rank sum test, two tailed). The values are presented as mean ± S.D. n = number of observations and t = time.

The adrenaline concentration used (1 µM) is of the same magnitude as those found in plasma from "stressed" fish (NAKANO & TOMLINSON 1967, WAHLQVIST 1982). However, a conceivable effect on the adrenergic system may not become apparent at those concentrations of adrenaline in conjunction with the tissue concentrations of PCB obtained (Table 3).

TABLE 3. PCB content in different tissues after feeding Aroclor

Type of exp.	ppm PCB per wet weight		
	liver	muscle	gill filament
Control	0.055 n=1	0.125 n=2	-
PCB	33 n=1	70 n=2	5.9 n=1

PCB content in different tissues 38 days after feeding 0.173 g Aroclor 1254 per os. PCB is analysed with gas chromatography. n=number of observations.

OLOFSSON & LINDAHL (1979) found with the rotatory-flow technique an impaired capacity in Cod (Gadus morrhua) four days after oral intubation of ten ppm PCB resulting in 1.8 ppm PCB in wet tissue. JOHANSSON et al. (1972) found an increased level of muscle glycogen in Brown trout (Salmo trutta) 40 days after oral intubation of PCB resulting in three ppm in wet tissue. FÖRLIN et al. (1979)

also found increased levels of muscle glycogen in males of Platichthys flesus 82 days after oral intubation. Their highest experimental dose of PCB was one tenth of ours.

The necessity of parameters for viability is clearly stated by PÄRT & SVANBERG (1981) and PÄRT et al. (1982a, 1983). In this study the steadiness of To_2, ventral pressure and sodium uptake from the external medium have been used as parameters for viability (PÄRT & SVANBERG, 1981; PÄRT et al. 1982a). We found no viability differences between the two groups (Tables 1 and 2).

In mammals adrenergic stimulation mobilizes glycogen in white muscle (VILLA et al. 1980). White muscle consists mainly of fast twitch fibres and use glycogen as the main energy source.

WENDT (1965, 1967) found a rapid decrease in muscle glycogen in response to stress in Baltic salmon (Salmo salar). NAKANO & TOMLINSON (1967) found that this decrease of muscle glycogen is correlated to an increase of the level of adrenaline in the blood in Rainbow trout. If PCB inhibits the adrenaline mediated muscle glycogen mobilization, this would result in an increasing content of muscle glycogen in PCB exposed fish.

A significant increase in muscle glycogen is reported by FÖRLIN et al. (1979) and JOHANSSON et al. (1972) in fish exposed to PCB. However, in our experiments, no significant difference in the content of muscle glycogen was found between the PCB exposed and the control group (Table 4).

TABLE 4. Muscle glycogen content after PCB feeding

	Level of muscle glycogen mg/wet weight		
Type of exp.	males	females	total
Control	2.16±0.74 * (ns) n=6	1.16±0.55 (ns) n=8	1.44±0.64 (ns) n=25
PCB	1.86±0.55 (ns) n=11	1.08±0.60 n=11	1.47±0.66 n=25

Muscle glycogen content from Feb.-March in white muscle obtained from freeze clamped samples. $P<0.05$ (Wilcoxon rank sum test two tailed). The values are presented as mean ± S.D. n = number of observations.

However, a significantly higher content of muscle glycogen was observed in males than in females of the control group. In the PCB exposed group (Table 4) the difference was not statistically significant.

The males but not the females in both groups were sexually mature according to the definition by FLUME (1978). This maturation difference has earlier been observed by WENDT (1965) who found a higher

glycogen content in precocious males than in immature males.

In a number of studies (e.g. FÖRLIN et al. 1979, FINGERMAN & RUSSEL 1980, HEINZ et al. 1980) PCB was found or suspected to disturb the hormone balance and/or the normal function of the regulating neurons. Whether the difference in muscle glycogen content between males and females in the two groups depends on such PCB effects was beyond the scope of this study.

No starvation effect on the levels of glycogen in the muscle were observed, although the fish were starved throughout the experimental period of 38 days. Such a stable glycogen level was also found by WENDT (1965), during Feb-March, in starved Baltic salmon.

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