Sensitivity of Fish ATPases to Polychlorinated Biphenyls

In spite of widespread concern about the possible hazards of polychlorinated biphenyls (PCBs)[1-11], little is known about the physiological action of these materials. We have therefore examined the effect of PCBs on the ATPase enzyme system, which is known to be sensitive to chemically similar compounds such as DDT[12-18].

It is known that PCB ('Aroclor 1242') will cause oedema and depress growth when fed to chicks in large amounts (200 and 400 p.p.m. in diet), the rate of steroid metabolism is increased in liver homogenates from birds dosed with PCBs and that the materials stimulate oestrogenic activity in the rat uterus[10,11]. We have examined the activity of the ATPase enzyme system in homogenized and fractionated tissues of the blue gill fish *Lepomis machrochirus*, because this system is sensitive to DDT and related compounds and because distinctive differences in effect have been found among cyclodiene-type chlorinated hydrocarbons.

Tissues were dissected, homogenized and fractionated according to the procedure reported by Koch[16]. The fraction used (B) contained mitochondria plus nerve endings. Each preparation was diluted appropriately, quick frozen in liquid nitrogen and stored at −20° C. ATPase activities were determined essentially according to the enzymatic procedure described before[19-21]. Proteins were determined by the method of Lowry *et al.*[22]. Reaction mixtures used are given in Table 1.

Table 1 Percentage Inhibiton of Mg^{2+} ATPase from Muscle of Blue Gill Fish by PCB Preparations*

Concentration (p.p.m.)	Percentage inhibition 'Aroclor 1221'	'Aroclor 1254'	'Aroclor 1268'	'Aroclor 5460'
0.03	18.7	20.6	37.2	31.9
0.33	29.9	30.6	47.1	42.9
3.33	65.4	73.1	60.3	—
10.00	80.4	84.3	67.5	—

A 3 ml. reaction mixture contained compounds at the following concentrations: 4.3 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 125 mM imidazole buffer (pH 7.5), 0.19 mM NADH, 0.5 mM PEP, 0.02% BSA, approximately 9 U pyruvate kinase and 12 U lactic dehydrogenase, and 100 µl. homogenate fraction. Absorbance changes were measured at 340 nm for 15 min using a Beckman DU spectrophotometer with temperature controlled at 27° C in reaction mixture. When used, ouabain was present at 0.85 mM. Total ATPase activity was determined in the presence of Na^+, K^+ and Mg^{2+}. Mg^{2+} ATPase activity was measured using same mixture plus ouabain. Na^+-K^+ ATPase activity is total activity minus Mg^{2+} ATPase activity[19,21]. Ethanol was used as solvent for the 'Aroclor' except 'Aroclor 1268', where acetone was used. Between 1 and 5 µl. of the 'Aroclor' solutions was added to the enzyme reaction mixture by slowly releasing the solution from a Hamilton microsyringe into a rapidly stirred reaction mixture.

* Each value represents the mean from three separate preparations. Specific activities (µmol Pi mg^{-1} protein h^{-1}; mean ± s.e.) for untreated muscle Mg^{2+} ATPase and Na^+-K^+ ATPase were 57.1 ± 2.2 and 2.3 ± 0.8 respectively. Low specific activity of Na^+-K^+ ATPase in muscle made accurate determinations of inhibition very difficult.

Three PCB preparations, 'Aroclor 1221', 'Aroclor 1254' and 'Aroclor 1268', and one polychlorinated terphenyl, 'Aroclor 5460' (Monsanto) were used. The last two digits of the name give the percentage chlorination of each compound, but the exact composition is not available. All compounds inhibited ATPases in low concentrations (less than 1 p.p.m.) with the most pronounced inhibition on Mg^{2+} ATPase of muscle (Tables 1 and 2). The higher sensitivity of muscle Mg^{2+} ATPase to PCB compounds is consistent with the high sensitivity of this enzyme to DDT[15-18].

'Aroclor 1254' was the most effective inhibitor of ATPases, especially Mg^{2+} ATPase, from all tissues (Tables 1 and 2). 'Aroclor 1268', an effective inhibitor of muscle Mg^{2+} ATPase (Table 1), differed from the other PCB compounds in having minimal effects on Mg^{2+} ATPase activity from other tissues (Table 2). 'Aroclor 5460' seemed to be intermediate between 'Aroclor 1268' and the other 'Aroclor' compounds in its effect on Mg^{2+} ATPase activity (Table 2). The variation between tissues, most prominent with 'Aroclor 1268', indicates that the characteristics of Mg^{2+} ATPase differ depending on tissue source. A 30–40% inhibition of Na^+-K^+ ATPase measured only on brain and kidney preparations was consistently determined for all PCBs.

Table 2 Inhibition of Mg^{2+} ATPase and Na^+-K^+ ATPase from Tissues of Blue Gill Fish

Concentration (p.p.m.)	Percentage inhibition * 'Aroclor 1221' Mg^{2+}	'Aroclor 1221' Na^+-K^+	'Aroclor 1254' Mg^{2+}	'Aroclor 1254' Na^+-K^+	'Aroclor 1268' Mg^{2+}	'Aroclor 1268' Na^+-K^+	'Aroclor 5460' Mg^{2+}	'Aroclor 5460' Na^+-K^+
(*A*) Brain								
0.03	0.3	4.6	4.2	16.9	0.4	29.4	2.3	26.9
0.33	8.4	10.5	0.7	19.1	+1.5†	27.6	2.9	26.3
3.33	7.2	20.4	27.7	41.5	+14.0	43.6	2.5	38.9
10.00	36.0	46.4	57.9	45.5	8.3	39.4	11.2	42.7
(*B*) Kidney								
0.33	7.4	15.1	1.3	35.2	0.0	25.4	0.0	22.3
3.33	5.0	24.3	33.4	45.9	+7.9	40.1	4.5	25.2
10.00	42.8	43.3	60.1	51.7	+4.8	48.0	+1.4	33.8
(*C*) Liver								
0.03	2.2		3.9		—		—	
0.33	2.6		19.4		+5.3		6.6	
3.33	2.9		24.3		+5.1		9.3	
10.00	35.8		53.0		+2.4		15.8	
16.70	45.9		74.1		6.2		—	

A, Specific activities for untreated brain, Mg^{2+} ATPase 25.2 ± 1.0, Na^+-K^+ ATPase 35.8 ± 2.1.

B, Specific activities for untreated kidney, Mg^{2+} ATPase 31.3 ± 1.3; Na^+-K^+ ATPase 42.0 ± 0.7.

C, Specific activities for untreated liver, Mg^{2+} APTase 28.5 ± 3.0; Na^+-K^+ APTase 2.8 ± 0.8; low specific activity of Na^+-K^+ ATPase in liver made accurate determinations of inhibition very difficult.

For reaction conditions see Table 1 except that temperature was 37° C.

* Each value represents the mean from three separate preparations.

† Plus values represent percentage activation of enzymatic activity.

In general, the PCB preparations do not reveal any obvious relationships between percentage chlorination and inhibitory effects. But the differences in action of 'Aroclor 1268' on Mg^{2+} ATPase from different tissues and the similarities in effects between PCBs and DDT require further study.

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Effect of Pregnancy on the Isoantibody Response in Rabbits

When an Rh-negative woman becomes pregnant for the first time with an Rh-positive foetus, the immune response to Rh-positive foetal red cells entering the maternal circulation is usually manifested by the appearance of antibodies after delivery or by priming without detectable antibodies, which only appear during a subsequent Rh-positive pregnancy[1]. The appearance of antibodies during the first pregnancy, in the absence of any known prior stimulus with Rh-positive blood, is not as rare as was formerly thought. A minimum of 1% of such women can be found to have antibodies at the end of the first preganancy, and they appear during the later months of ABO-compatible pregnancies[2].

Although the passage of foetal cells into the maternal circulation is most frequent and intense as the result of labour, it also occurs during pregnancy particularly during the third trimester[3]. The relative infrequency of antibodies at the end of the first pregnancy and the frequent delay in their first appearance after delivery has led to the suggestion that women possess a diminished immune responsiveness to foetal red cell antigens during pregnancy[3,4], but the only direct experimental evidence is that of Merritt and Galton[5], who found that the antibody response of three different species of experimental animal to sheep red blood cells, bacterial endotoxin and bovine γ-globulin respectively, was the same in pregnant as in non-pregnant animals.

To obtain further information on this point, but using an iso-antigenic system, random-bred Californian virgin female Hg^A-negative rabbits were mated and injected intravenously with 2×10^9 Hg^{A^p} red cells at varying intervals after mating. One blood donor was used throughout the experiment. In an initial group of rabbits (group 1) the injections were given 14 days after mating, and in those that became pregnant the interval between the stimulus and delivery of the litters was found to be 17–20 days. A second group of rabbits (group 2) was injected at approximately 21 days after mating, the interval between stimulus and delivery being 9–13 days. Samples of venous blood were obtained from each rabbit at the beginning of the experiment and at 10, 20 and 30 days after the injection of red cells. The sera were tested for haemagglutinating anti-Hg^A antibodies using papainized Hg^A-positive red cells.

Table 1 Anti-Hg^A Response in Rabbits Injected 14 days after Mating

	Pregnant rabbits		Non-pregnant rabbits	
	20 days after stimulus	30 days after stimulus	20 days after stimulus	30 days after stimulus
Anti-Hg^A present	10 (55.6%)	10 (55.6%)	7 (41.2%)	9 (53.0%)
Anti-Hg^A absent	8	8	10	8
Totals	18	18	17	17

Tables 1 and 2 give the results of testing for antibodies in the two groups of rabbits, which are divided into those that became pregnant as a result of the mating and those that did not, the latter acting as controls. (The overall incidences of anti-Hg^A in the control rabbits was 51% which is very close to the 56% incidence found in a group of non-mated control animals in a previous study[6].) The incidence of positive tests for antibodies is given both for tests taken at the time when the pregnant rabbits reached the end of gestation and for tests taken 30 days after the stimulus. The latter are included because the pregnant state might have influenced not only the development of antibodies up to the point of delivery, but also their appearance in the post-gestational period. Previous experience[6] had shown that if antibodies were to develop they had done so by 30 days after the stimulus.

Table 2 Anti-Hg^A Response in Rabbits Injected 21 Days after Mating

	Pregnant rabbits		Non-pregnant rabbits	
	10 days after stimulus	30 days after stimulus	10 days after stimulus	30 days after stimulus
Anti-Hg^A present	7 (50%)	12 (85.7%)	11 (32.4%)	17 (50%)
Anti-Hg^A absent	7	2	23	17
Totals	14	14	34	34

In group 1, samples taken 20 days after the stimulus, that is at a time when pregnant rabbits reached the end of gestation, showed that 41.2% of non-pregnant rabbits had antibodies while these were detected in 55.6% of pregnant animals. The difference is not significant. During the subsequent 10 days two further non-pregnant rabbits developed antibodies, the final incidences in the two groups becoming almost identical. In group 2, tests or samples taken 10 days after the stimulus, that is, at a time when the pregnant rabbits were at the end of their gestation period, showed that 32.4% of non-pregnant rabbits had antibodies, compared with 50% of pregnant animals. During the subsequent 20 days a further eleven developed antibodies, the final incidences being 50% for non-pregnant and 85.7% for pregnant animals.

There is thus no evidence that during pregnancy the rabbit showed any diminished unresponsiveness to Hg^A-positive red cells. The peak titres of antibody reached in responding rabbits varied from 1 : 1 to 1 : 256, and there was no difference in the distribution of titres between pregnant and non-pregnant rabbits. There is a suggestion of an increased responsiveness

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