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Effect of Polychlorinated Biphenyls (Phenoclor DP6) on *In vitro* Amino Acid Incorporation Into Liver Proteins*

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The administration of polychlorinated biphenyls (PCBs) to rats produces a marked increase in liver microsomal drug-oxidizing enzymes (LITTERST et al. 1972, BRUCKNER et al. 1973, GRANT and PHILLIPS 1974). ZEPP et al. 1974 have shown that this enhanced activity has functional signifiance as indicated by increased rate of drug metabolism in vivo. A similar activation of these enzymes occurs after application of various lipids soluble drugs (SLADEK and MANNERING 1969, MASTEN et al.1975, NELSON and KEARNEY 1977). SHUSTER and JICK (1966) suggested that the increased microsomal enzyme activity reflects an increase in the rate of synthesis. In vitro experiments demonstrate that each homogenate fraction used (mitochondria, microsomes, supernatant) contributes to the increased incorporation rate observed in the preparations from rats treated with methylcholanthrene (GELBOIN and SOKOLOFF 1961). More recently NISHIZUMI (1970) and ALLEN et al. (1974) have shown by electron microscopy that chronic administration of PCBs causes a proliferation of smooth membrane fraction of liver endoplasmic reticulum.

In order to examine the mechanism of proteosynthesis induction by PCB, a study was undertaken to determine the effect of Phenoclor DP_6 ** chronic administration on the in vitro rate of amino acid incorporation into protein by purified polysomal fraction.

METHODS

Male Sprague Dawley rats, weighing 150 g, were divided into 2 groups of 5 rats each and were fed diet containing 0 and 10 ppm (wet weight) of Phenoclor DP_6 incorporated into arachid oil. Rats had been fed on experimental diets for 4 weeks; 12,6 mg $\pm$ 1,43 of DP_6 were ingested during intoxication time (45 mg/kg body weight) by treated rats. Animals were fasted 16 h before they were killed. The rats were decapited and the liver

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and skeletal muscles of rear legs were removed and homogenized into chilled sucrose solution 0,25 M, pH 7,5. The liver fractions were separated by differential centrifugation (NARBONNE 1978). Post-nuclear supernatant was treated with sodium deoxycholate (D.O.C.) and the post-mitochondrial supernatant was layered in 10 ml aliquots over 4 ml of medium sucrose 0,35 M. The polysomes obtained after 120 min. at 105 000 x g were suspended in medium C (Tris 0,045 M, pH 7,5, KCl 0,12 M, $MgCl_2$ 0,0075 M, NaCl 0,075 M). The incubation mixture (final volume 1 ml) contains 0,3 ml of polysome suspension ($\simeq$ 1 mg RNA/ml), 0,3 ml of supernatant fluid ($\simeq$ 20 mg Protein/ml), 0,2 ml of energetic mixture (ATP 1 mM, GTP 0,6 mM, Phospho-enol-pyruvate 10 mM, Pyruvate kinase 30 mg/l), 0,1 ml of L lysine U^{14} C(5 μCi/ml), 0,1 ml of amino acid mixture (1 μM/ml) according with SCHMITT et al.(1968). All samples were prepared in duplicate. Incubation was performed at 37° C for 30 min. The reaction was stopped with 1 ml of 10 % trichloracetic acid. The proteins were washed and counted as previously described (NARBONNE 1978). Proteins were determined by the method of LOWRY et al. (1951). RNA was determined by the method of SCHMIDT and THANNHAUSER (1945) modified by FLECK and MUNRO (1962).

Two cell-free amino acid incorporating systems were used. One consisted of liver polysomes and cell-sap. In other liver polysomes were replaced by skeletal muscle polysomes. In each system, experiments were made by crossing-over incubations.

RESULTS AND DISCUSSION

Table 1 shows the effect of DP_6 treatment on total liver weight, total liver proteins, liver microsomal proteins and liver microsomal RNA. These results confirm a previous work. DAUBEZE (1977) and NARBONNE and DAUBEZE (1977) showed an increase in microsomal liver proteins and a stability of total liver RNA after DP_6 treatment.

Table 2 shows that administration of DP_6 induced an increase in the rate of amino acid incorporation into proteins (+ 40 % system I). When treated supernatant systems were incubated with control polysomes, amino acid incorporation was enhanced (+ 289 % in system I, + 202 % in system II). DP_6 treated polysomes (from liver and muscle) were less active when incubated with control supernatant (- 45 % and - 29 %, respectively) than control polysomes.

Table 3 shows that <u>in vitro</u>, amino acid incorporation was unaffected by addition of DP_6 solubilized in incubation medium with dimethylsulfoxyde. These results indicate that only the postpolysomal supernatant fraction contributes to the increased amino acid incorporation into proteins, suggesting an important rôle of messenger RNA and t RNA synthetase system present in this fraction. The liver polysomal fraction capacity

TABLE 1

Effect of Phenoclor DP_6 treatment on Rat liver weight, liver total proteins, liver microsomal proteins and liver polysomal RNA levels[a]

	Controls (5)[c]	DP_6-treated(5)	Difference %	P[b]
Liver weight (g/100 g body wt)	3.38 ± 0.84	5.11 ± 0.41	+ 51	< 0.01
Total protein (mg/g liver)	126 ± 6	147 ± 13	+ 16	< 0.05
Microsomal protein (mg/g liver)	29.3 ± 1.89	41.5 ± 3.94	+ 41	< 0.01
Polysomal RNA (mg/g liver)	0.94 ± 0.061	0.87 ± 0.058	- 7	< 0.20

a Results are expressed as mean ± SE

b Significance was juged by the Student test t

c In brackets the number of experiments

TABLE 2

Effect of Phenoclor DP_6 treatment on amino acid incorporation in vitro

Polysomes	Supernatants	Specific activity[a] (dpm/mg RNA)	Difference[b] %	P[c]
		System I[d]		
Control	Control	1 591 ± 178		
DP_6 treated	DP_6 treated	2 225 ± 382	+ 40 (10)	< 0,001
DP_6 treated	Control	868 ± 117		
Control	DP_6 treated	3 378 ± 432	+ 289 (10)	< 0,001
		System II[e]		
Control	Control	1 603 ± 160		
DP_6 treated	DP_6 treated	4 945 ± 481	+ 208 (10)	< 0,001
DP_6 treated	Control	1 124 ± 134		
Control	DP_6 treated	3 403 ± 333	+ 202 (10)	< 0,001

a Results are expressed as mean ± SE

b In brackets the number of experiments

c Significance was juged by the student test t

d In system I the polysomal and supernatant fractions were prepared from liver

e In system II the polysomal fraction was prepared from skeletal muscle (rear legs) the supernatant fraction was prepared from liver

TABLE 3

In vitro effect of Phenoclor DP_6 on amino acid incorporation on Rat liver cell-free system [d]

Substance added in flask D.M.S.O.[c] (%)	DP_6 (ppm)	Number of experiments	Specific activity[a] (dpm/mg RNA)	Compared groups		Difference %	P[b]
0	0	4	1 120 ± 229				
4	0	4	927 ± 200	Control	DMSO	- 17	> 0.05
4	100	4	1 055 ± 205	DMSO	DP_6	+ 13	> 0.05
4	500	4	1 077 ± 123	"	"	+ 16	> 0.05
4	2 000	4	1 078 ± 160	"	"	+ 16	> 0.05
4	5 000	4	959 ± 235	"	"	+ 3.4	> 0.05

a Results are expressed as mean ± SE
b Significance was juged by the student test t
c Dimethyl Sulfoxyde
d Polysomal and supernatant fractions were prerared from control rat liver

was little decreased by DP_6 treatment while incorporation remains more elevated than that of controls.

The present study demonstrates that chronic ingestion of DP_6 stimulates amino acid incorporation into liver proteins and therefore suggests an accelerated rate of protein synthesis. These results, combined with morphological observations (ALLEN et al. 1974), indicate that the polysomal fraction (bond to rough reticulum) remains stable in quantity while specific activity was little depressed by DP_6 treatment. The activating factor was located in post-polysomal supernatant.

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