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Effect of Hexachlorobenzene (HCB) on the Activity of some Enzymes from *Tetrahymena pyriformis*

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HCB is a very persistent environmental contaminant which is widely distributed in the ecosystem. High concentrations of this compound have been found mainly in organisms at the end of food chains, for instance in predatory birds (VOS et al. 1968, KOEMAN et al. 1969b), fish (KOEMAN et al. 1969a), human milk (ACKER and SCHULTE 1970, SIYALI 1973), adipose tissue (ACKER and SCHULTE 1970, BRADY and SIYALI 1972, CURLEY et al. 1973) and blood (SIYALI 1972, ZEMAN et al. 1971). Even in cereals and other plant material HCB residues have been detected (STIJVE 1971).

Outbreaks of cutaneous porphyria due to HCB, involving several thousand cases, have occurred in Turkey since 1956 (CAM and NIGOGOSYAN 1963) and short-term feeding experiments have shown that HCB disturbed porphyrin metabolism (OCKNER and SCHMID 1961, DE MATTEIS et al. 1961, VOS et al. 1971). The mechanism by which HCB induces porphyria, however, is not known but from studies of GRANICK (1967) and RAJAMANICKAM et al. (1972) this might be due to an induction of δ-aminolevulinic acid synthetase, the first enzyme of the biosynthetic pathway of porphyrins.

Studies with polychlorinated biphenyls (PCB) have shown that these environmental pollutants can induce hepatic microsomal enzymes in rats (LITTERST and VAN LOON 1972, BRUCKNER et al. 1973, JOHNSTONE et al. 1974) and channel catfish (HILL et al. 1976) and it is fairly well established that chlorinated hydrocarbon insecticides such as DDT are powerful inducers of microsomal enzymes (HART and FOUTS 1963, STREET 1964, MORELLO 1965, ABOU-DONIA and MENZEL 1968). Recently it has been shown that HCB is also a potent inducer of hepatic microsomal drug metabolizing enzymes and of cytochrome P-450 (WADA et al. 1968, GRANT et al. 1974, STONARD and NENOV 1974, LISSNER et al. 1975, MEHENDALE et al. 1975, CARLSON and TARDIFF 1976). Little is known, however, about the effect of HCB on the activity of enzymes other than microsomal drug metabolizing.

The present paper presents evidence that HCB affects the activity of different enzymes in Tetrahymena pyriformis.

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MATERIALS AND METHODS

Tetrahymena pyriformis Wh 14 from the Pringsheim algae collection, University of Göttingen, Germany, was transferred from stock cultures to 500-ml-Erlenmeyer flasks containing 250 ml of nutrient solution as described by GEIKE (1969) and grown under continuous stirring with a magnet stirrer arrangement in an incubator for 3 days at 30°C. After this time, when the culture had produced a dry matter of about 100 mg, the appropriate amount of HCB in acetone solution (1.0 ml) was added, and the culture was incubated for another 7 days; the controls received the same amount of acetone. After incubation the cells were harvested by centrifugation for 10 min at 3000 x g, washed twice with 0.05 M K-phosphate buffer pH 7.0 and finally were suspended in 0.05 M phosphate buffer pH 7.0, containing 0.01 M cysteine to give a total volume of 6.0 ml. The suspension was subjected twice to a freezing-thawing-process, then homogenized in a Potter-Elvehjem homogenizer for 5 min, and finally centrifuged for 15 min at 25000 x g. Aliquots of the clear supernatant were used for the determination of enzyme activities. The protein content of the crude enzyme extract was determined by the method of WARBURG and CHRISTIAN (1941), the activity of δ-aminolevulinate dehydratase was determined by measuring the formation of porphobilinogen according to the procedure of MAUCERALL and GRANICK (1956). The standard reaction mixture contained 100 mM phosphate buffer pH 8.0, 1 mM $MgCl_2$, 1 mM cysteine, neutralized with NaOH, and 10 mM δ-aminolevulinic acid (ALA), neutralized with NaOH. Incubation was 30 min at 37°C. Specific activity is defined as micromoles of porphobilinogen formed per milligram of protein in 1 minute. The activity of the other enzymes studied was determined by the methods described by BERGMEYER (1974).

RESULTS AND DISCUSSION

The effect of HCB on the activity of 8 enzymes was studied in experiments with 0, 0.001, 0.1 and 1.0 ppm HCB in nutrient solution which contained 0.4 % acetone after addition of this compound. The data presented here are drawn from one experiment and are representative of a further five replicates. As can be seen from Table 1 a concentration of 0.001 and 0.1 ppm HCB in nutrient solution compared to control leads to an increase in the activity of most of the enzymes studied, the only exception being GPT the activity of which was decreased by

TABLE 1

Effect of HCB exposure of Tetrahymena pyriformis on the activity of some enzymes

HCB concentration (ppm)	Specific activity of the enzymes (mU / mg protein)							
	ALA-D	HK	PK	GOT	GPT	ICDH	GDH	MDH
Control	5.6	93.5	93.9	75.7	86.0	151.7	10.2	440.1
0.001	5.7	91.6	93.7	167.6	48.7	167.9	19.7	3041.7
0.100	7.7	120.2	113.0	222.1	46.2	231.5	12.4	3219.7
1.000	2.8	89.7	92.0	72.5	6.3	196.3	6.1	1811.3

Abbreviations used: ALA-D δ-aminolevulinate dehydratase EC 4.2.1.24;
GDH glutamic dehydrogenase EC 1.4.1.2; HK hexokinase EC 2.7.1.1;
MDH malate dehydrogenase EC 1.1.1.37; PK pyruvate kinase EC 2.7.1.40;
GOT glutamic-oxaloacetic transaminase EC 2.6.1.1;
GPT glutamic-pyruvic transaminase EC 2.6.1.2;
ICDH isocitrate dehydrogenase EC 1.1.1.41

increasing the HCB concentration. As can further be seen a concentration of 0.1 ppm was most effective in inducing the enzymes studied under the experimental conditions applied in this study while 1.0 ppm in most cases reduces enzyme activity below control levels.

The rise in ALA-D supports the findings of those workers who noted the induction of porphyria and an increase in the amount of cytochrome P-450 by HCB. This increase in ALA-D may reflect an elevated synthesis of ALA by ALA synthetase as was noted by GRANICK (1967) and RAJAMANICKAM et al. (1972).

Hexokinase (HK) initiates the utilization of glucose from the nutrient solution by the organism and, together with pyruvate kinase (PK), may be regarded as a catabolic enzyme. Although there was no apparent change in the activity at a dose level of 0.001 ppm the rise of these two enzymes due to an exposure to 0.1 ppm HCB reflects an increase in metabolic activity leading to elevated substrate levels metabolized via the Krebs cycle.

A product of most transaminase reactions is glutamate which is translocated into the mitochondria by a specific carrier-system. While GOT is increased by an exposure of Tetrahymena pyriformis to concentrations of 0.001 and 0.1 ppm HCB the activity of GPT is depressed by HCB in nutrient solution. An initial increase in GOT activity was also noted in liver and serum of rats exposed to HCB while GPT activity was decreased (IWANOW et al. 1973). Glutamic dehydrogenase (GDH) which like GOT is found both in soluble cytoplasm and mitochondria is increased after exposure to HCB at dose levels of 0.001 and 0.1 ppm, a concentration of 0.001 ppm being most effective. Part of these different effects of HCB on the activity of these glutamate metabolizing enzymes might be explained by the different localisation of these enzymes within the cell, for the activity of the two mitochondrial enzymes, malate dehydrogenase and isocitrate dehydrogenase, studied here was also increased by 0.001 and 0.1 ppm HCB with maximum activity at a dose level of 0.1 ppm.

Apart from ALA-D all enzymes studied directly or indirectly give rise to reducing power which may be used in energy production or microsomal drug metabolism. From experiments in this laboratory, however, it is known that oxygen consumption of Tetrahymena pyriformis is depressed by an exposure to HCB. Therefore, it may be suggested that most of the reducing power produced is used in drug metabolism or biosynthetic pathways. In experiments where Tetrahymena was exposed to HCB from the inoculation of the cultures a severe loss of total nitrogen and carbohydrates from the cells was noted (GEIKE and PARASHER 1976). This effect might be attributed to damage to the cell membranes observed in experiments

with Chlorella (PARASHER et al. 1978) which enables a leakage of cellular material like amino acids, proteins and soluble carbohydrates. The decrease in enzyme activity compared to control cells observed at a dose level of 1.0 ppm HCB might reflect this damage to cell membranes. Although, compared to maximum activity, a decrease in the activity of mitochondrial enzymes can also be observed, the level is higher than that found in controls (MDH, ICDH) or is only slightly reduced. Again, this may be attributed to an early damage to cell membranes by HCB and damage to the mitochondrial membrane occurring relatively late during the experiment.

SUMMARY

Tetrahymena pyriformis Wh 14 was grown in Erlenmeyer flasks under continuous stirring at 30°C for three days. After the culture had produced dry matter of about 100 mg HCB was added in acetone at a dose level of 0, 0.001, 0.1 and 1.0 ppm to the culture and incubated for another 7 days. At a dose level of 0.001 ppm the activity of δ-aminolevulinate dehydratase, hexokinase, and pyruvate kinase remained unaffected but was increased for glutamic-oxaloacetic transaminase, glutamic dehydrogenase, isocitrate dehydrogenase, and malate dehydrogenase while 0.1 ppm HCB increased the activity of all enzymes studied, the only exception being glutamic-pyruvic transaminase, the activity of which was depressed by HCB exposure. A concentration of 1.0 ppm HCB depressed the activity of most of the enzymes below control values with the exception of the two mitochondrial enzymes, MDH and ICDH, studied here.

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