Effect of Dieldrin on the Stability of Lysosomes in the Rat Liver[1]

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Lysosomes are known to be responsible for the digestive processes in the cell, mainly because of the presence of various digestive enzymes in these subcellular organelles (BARRETT, 1972). Stability of lysosomes is affected by various compounds. Vitamin A (WEISSMANN et al., 1963) and carbon tetrachloride (DIANZANI, 1963) have been reported to increase in vivo the amount of free lysosomal enzymes in liver. Carbon tetrachloride (ALPERS and ISSELBACHER, 1967), ultraviolet irradiation (DESAI et al., 1964) and gamma-irradiation (TAPPEL et al., 1963) cause damage to lysosomes in vitro. Though dieldrin is known to affect various metabolic processes associated with lipid (KOHLI et al., 1975), carbohydrate (BHATIA et al., 1973) and protein metabolism (KOHLI and VENKITASUBRAMANIAN, 1975), there are no reports available on the effect of dieldrin on the lysosomal enzymes. Hence, study was undertaken to investigate the effect of dieldrin on the stability of lysosomes. The activity of bound and free lysosomal enzymes has been assayed in control and dieldrin-treated rats.

MATERIALS AND METHODS

Wistar strain male rats 100-120 g were used. Animals were divided into control and experimental group with five animals in each group. The experimental animals were fed orally with a single oral dose of dieldrin

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(30 mg/kg body weight) dissolved in groundnut oil. The control animals received only the vehicle oil. The rats were fasted for 22-24 hr and had free access to water during fasting. After fasting, rats of both the groups were sacrificed, their livers removed and processed as follows.

Liver was minced and homogenized in 0.25 M sucrose solution using Potter-Elvehjem homogenizer. The nuclear fraction was removed by centrifuging the homogenate for ten minutes at 1000 x g in an International refrigerated centrifuge at 4°C. The supernatant was centrifuged at 6,500 x g for ten minutes under the same conditions. The supernatant thus obtained was centrifuged at 12,000 x g for 30 minutes. The pellet was resuspended in 0.25 M sucrose and centrifuged at 12,000 x g for 5 minutes. The pellet so obtained was used to assay the bound activity of lysosomal enzymes in presence of Triton X-100 (1 mg/ 0.1 ml). The 12,000 x g pooled supernatant was spun at 100,000 x g for 60 minutes in a Beckman ultracentrifuge (Model L2-65B). The supernatant thus prepared was tested for the free activity of lysosomal enzymes without the use of Triton X-100.

Cathepsin, acid ribonuclease, aryl sulphatase and acid phosphatase were assayed by the method of ANSON (1939), THYAGARAJAN et al.(1974), ROY (1953) and LOWRY et al. (1951) respectively.

RESULTS AND DISCUSSION

The effect of dieldrin on the activity of bound and free lysosomal enzymes, viz., cathepsin and acid ribonuclease are presented in Table I and that of acid phosphatase and aryl sulphatase in Table II. Administration of dieldrin increases the activity of free acid ribonuclease, cathepsin and acid phosphatase while no effect is observed on the activity of free aryl sulphatase. The results show that dieldrin labilizes the

TABLE I

Effect of a single oral dose of dieldrin (30 mg/kg body weight) on the activity of cathepsin and acid ribonuclease in the rat liver

Parameter	Enzyme activity Units*/mg protein	
	Cathepsin	Acid ribonuclease
Bound activity		
Control	113±4.8	363±18
Experimental	97±5.5	325±21
	$p > 0.05$	$p > 0.05$
Free activity		
Control	12.2±0.37	50±4
Experimental	22.4±1.12	102±17
	$p < 0.001$	$p < 0.02$
Per cent free activity		
Control	9.81±0.42	12±0.8
Experimental	18.8 ±0.45	24±2.5
	$p < 0.001$	$p < 0.01$

The values are mean ± SE of the results obtained from five animals. Data have been statistically evaluated and $p \leq 0.05$ is considered statistically significant.

*Cathepsin: One enzyme unit is expressed as the amount of enzyme required to liberate one nmole of tyrosine in 30 minutes under the assay conditions.

*Acid ribonuclease: One unit of enzyme is expressed as the amount of the enzyme required to liberate one nmole of adenine in 30 minutes under the assay conditions.

TABLE II

Effect of a single oral dose of dieldrin (30 mg/kg body weight) on the activity of acid phosphatase and aryl sulphatase in the rat liver

Parameter	Enzyme activity Units*/mg protein	
	Acid phosphatase	Aryl sulphatase
Bound activity		
Control	519±65	140±7.8
Experimental	393±27	134±12
	$p > 0.05$	$p > 0.05$
Free activity		
Control	101±3.6	15.6±0.8
Experimental	118±2.6	15.6±0.8
	$p < 0.01$	$p > 0.05$
Per cent free activity		
Control	17±2	10.10±0.74
Experimental	23.4±1.4	10.45±1.16
	$p < 0.05$	$p > 0.05$

The values are mean ± SE of the results obtained from five animals. Data have been statistically evaluated and $p \leq 0.05$ is considered statistically significant.

*Acid phosphatase: One unit of enzyme is expressed as the amount of the enzyme required to liberate one nmole of nitrophenol in 15 minutes under the assay conditions.

*Aryl sulphatase: One enzyme unit is expressed as the amount of the enzyme required to liberate one nmole of nitrocatechol in 30 minutes under the assay conditions.

lysosomes of rat liver, thus releasing the lysosomal enzymes. These observations are further supported by the report of ONIKIENKO (1963) that serum alkaline phosphatase activity was elevated in rats chronically exposed to dieldrin.

The role of the increased activity of the free lysosomal enzymes in dieldrin-treated rats is not clear at present. The increased activity of free cathepsin may or may not be responsible for increased breakdown of proteins because the mechanism of intracellular protein turnover is largely unknown. Circumstantial evidence has been presented to show lysosomal involvement in the turnover of intracellular protein (POOLE, 1975; MORTIMORE and NEELY, 1975). It has been reported that intracellular protein degradation is carried out mainly by lysosomes (DEAN, 1975). Increased protein synthesis, as demonstrated by the increased incorporation of radioactive leucine into liver protein of dieldrin-treated rats has been reported. No change was observed in the protein content (KOHLI and VENKITASUBRAMANIAN, 1975). This may be attributed to the increased activity of free cathepsin.

MELLORS et al. (1967) have demonstrated that lysosomes in vitro cause the swelling of mitochondria. Dieldrin has been observed to alter the morphology of mitochondria in the liver of rat treated with a similar dose of dieldrin as used in the present study (KOHLI, 1976). Mitochondrial matrix appeared electron dense, the cristae, few in numbers, were seen scattered in the mitochondrial matrix when examined under the electron microscope. Other workers have also demonstrated abnormal mitochondria in rats administered chlorinated insecticides (KIMBROUGH et al. 1971; HUTTERER et al. 1968). It is possible that the effect of dieldrin on mitochondrial morphology is mediated through lysosomal enzymes.

SUMMARY

The effect of dieldrin on the bound and free activity of cathepsin, acid ribonuclease, acid phosphatase and aryl sulphatase has been investigated. Administration of dieldrin increased the activity of free cathepsin, acid ribonuclease, and acid phosphatase in rat liver whereas it did not affect the activity of aryl sulphatase. This indicates that dieldrin labilizes the lysosomes.

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