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Effect of Intratracheally Administered DDT and Endosulfan on Pulmonary and Hepatic Respiratory Cytochromes

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Oxidative phosphorylation is the process in which ATP is formed as electrons are transferred from NADH or $FADH_2$ to O_2 by a series of electron carriers (Hinkle and McCarty, 1978). The respiratory assembly carrying out this process is an integral part of the inner mitochondrial membrane. The oligomycin sensitive, Mg^{2+}-dependent ATPase catalyzes the synthesis of ATP when it is coupled to electron flow. This process is adversely affected by DDT and its analogs (Koch et al, 1971; Cutkomp et al, 1971; Khan and Cutkomp, 1982). Byczkowski (1973, 1977) and Byczkowski et al. (1978) have reported stimulation of ATPase activity in rat liver mitochondria and they proposed DDT as an uncoupler of oxidative phosphorylation. On the other hand there are several reports dealing with insect models showing DDT as inhibitor of oxidative phosphorylation (Gregg et al, 1964; Ela et al. 1970; Waddill and Keeley, 1971).

The pesticides DDT (1,1'-(2,2,2-trichloroethylidene)-bis(4-chlorobenzene) and endosulfan (1,4,5,6,7,7-hexachloro-5-norbornene-2,3-di-methano cyclic sulfite) are chemically different and have different metabolic pathways in the animal systems (Peterson and Robinson, 1964; Gupta and Gupta, 1979). NADH supplies electrons for mitochondrial electron transport chain (Hinkle and McCarty, 1978). In addition NADH also generates NADPH by an energy dependent trans-hydrogenase system (Hoek and Brenster, 1974). The activity of NADH oxidase would limit the flow of electrons through electron carriers. Pardini et al. (1980) have recently reported that DDT, DDE (1,1'-(2,2-dichloroethenylidene)-bis(4-chlorobenzene) and TDE (1,1'-(2,2-dichloroethylidene)-bis (4-chlorobenzene) are inhibitors of heavy beef heart mitochondrial NADH oxidase and succino-oxidase enzyme systems. They have also suggested that DDE and TDE inhibit the electron transport on the substrate site of the cytochrome c.

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Lung comes first in contact with atmospheric pollutants. Few studies done on the effect of insecticides on animal mitochondrial electron transport system have used oral route of insecticide administration.A translocation of substances deposited in lungs has been observed to extrapulmonary organs (Holt, 1980). Thus in view of the paucity of information on the effect of insecticides given through pulmonary route on mitochondrial respiratory cytochromes, we have studied the effects of intratracheally administered DDT and endosulfan on NADH oxidase activity and cytochromes of lung and liver mitochondria of rats.

MATERIALS AND METHODS

Male wistar strain rats (weighing 200 to 250 g) were used in this investigation. The animals were fed Hind Lever Diet (Hindustan Lever Ltd.,Bombay, India) ad-libitum and had free access to water all the time.The rats were divided into following three groups and given the treatments shown below.

Rats of Group A were given DDT intratracheally(5mg/100g body weight) for three consecutive days. Rats of Group B were given endosulfan intratracheally(1mg/100g body weight) for three consecutive days. Rats of Group C were given only the vehicle solution intratracheally for the same period.

The insecticides were dissolved in ground nut oil and were administered through a cannula. Twenty four hours after the administration of last dose of insecticide rats were fasted over night and anaesthetized with sodium pentobarbitol (Abbott laboratories, Bombay, India, 6mg/100 g body weight given i.p.). They were secured on a board with rubber bands and the abdominal and thoracic cacities opened. The lungs were perfused through right ventricle with ice-cold physiological saline. After perfusion the lung and liver were removed, immersed in ice-cold saline, cleaned and weighed. A 20 percent homogenate of lung and liver were made in ice-cold 154 mM KCl; 50 mM Tris-HCl buffer, pH 7.4. Mitochondria were isolated by the method of Ritter and Malejka-Giganti (1982). The mitochondria were suspended in a known volume of 10 mM potassium phosphate, 2mM dithiothretol (Sigma Chem, Co. st. Luis), 2 mM $MgCl_2$ buffer, pH 7.4, containing 20 percent glycerol. All the operations were carried out at 0°-4°C. The protein was estimated by the method of Lowry et al. (1951). Mitochondrial suspension was assayed for cytochrome $a+a_3$, c and b spectrophotometrically according to the methods of Rieske(1967)and Gazzotti et al.(1980). In 2.5 ml of assay mixture,1.0ml enzyme protein (5-6 mg); 1.0% deoxycholate, 1.0 ml; 200 mM potassium phosphate buffer, pH 7.4, 0.45 ml;and

1 mM K_3 Fe(CN)$_6$ solution, 0.05 ml were added. The sample cuvette was stirred with few grains of $Na_2S_2O_4$ and the reduced-oxidized spectrum for cytochrome c and $a+a_3$ was recorded.Again the reference cuvette was stirred with few grains of TMPD (N,N-N,'N'-Tetramethylene-o-phenyl diamine) and recorded the spectrum for cytochrome b. The NADH oxidase activity in mitochondria were estimated according to the method of Mackler (1967).

RESULTS AND DISCUSSION

The air concentration of DDT has been reported to vary from 8.5 $\mu g/m^3$ to 140 mg/m^3 and was profoundly influenced by the use of DDT in that area (WHO Geneva 1979). The inhalation lethal dose of endosulfan has been reported about 350 mg/m^3 (Gupta 1976). The dose of DDT (50 mg/kg body weight) and endosulfan (10 mg/kg body weight) used in the present investigation is fairly low.

The effect of administration of DDT and endosulfan through intratracheal route on NADH oxidase of mitochondria and cytochromes of mitochondrial electron transport chain of lung and liver of rats is shown in Table 1 and 2. The effects of intratracheally administered insecticides on liver would indicate translocation of the insecticides as such or its metabolites to extrapulmonary organs. Mitochondrial protein and mitochondrial NADH oxidase activity of lung was not affected by either DDT or endosulfan as compared to the control (Table 1). Endosulfan significantly reduced the amount ofcytochrome b and cytochrome c, whereas DDT significantly increased the levels of cytochrome $a+a_3$ in lung mitochondria as compared to the control animals. The effects of these two insecticides on liver mitochondria shown in Table 2, depicts that the effects are more pronounced in liver as compared to lung. Both DDT and endosulfan enhanced liver mitochondrial protein as compared to control. Both DDT and endosulfan in liver significantly reduced the activity of mitochondrial NADH oxidase as compared to the control. Endosulfan administration significantly increased the level of cytochrome b and decreased the level of cytochrome $a+a_3$, where as DDT did not affect the level of these mitochondrial cytochromes in liver (Table 2).

These results indicate that the effects of intratracheally administered DDT and endosulfan are different in lung and extrapulmonary organs, and suggests a mobilization of the intratracheally deposited insecticides as such or its metabolite(s) to liver.Alterations in NADH oxidase activity would influence the availability of electrons for the mitochondrial electron transport and thus affect the generation of ATP (Pardini et al, 1980). Similarly the generation of NADPH by trans-hydrogenase reaction would also be affected by altered NADH oxidase

Table 1. Effect of intratracheally administered DDT and endosulfan on mitochondrial protein and NADH oxidase activity in rat lung and liver.

	Treatments	Lung	Liver
Protein	None	4.64 ± 0.18	9.04 ± 0.16
(mg/g tissue)	DDT	4.73 ± 0.05	10.00 ± 0.37*
	Endosulfan	4.10 ± 0.23	11.22 ± 0.36*
NADH oxidase	None	8.43 ± 0.49	20.32 ± 1.69
(nmol. NADH oxidized/ mg protein/min.)	DDT	8.34 ± 0.35	13.56 ± 0.76*
	Endosulfan	8.26 ± 0.74	13.05 ± 0.72*

Values are mean ± SE from six to eight rats in each group, The reaction mixture in 1.0 ml for assay of NADH oxidase activity contained 0.4 ml of 0.1% NADH solution; 0.005M,EDTA, pH 7.5; 0.2 M potassium phosphate buffer, pH 7.5; and diluted enzyme in 5% sucrose solution.

* Significantly different from control, $p < 0.05$.

Table 2. Effect of intratracheally adminstered DDT and endosulfan on respiratory cytochromes of rat lung and liver.

Cytochromes**	Treatment	Lung	Liver
Cytochrome b	None	0.430 ± 0.035	0.353 ± 0.029
	DDT	0.359 ± 0.017	0.394 ± 0.027
	Endosulfan	0.120 ± 0.001*	0.509 ± 0.012*
Cytochrome C	None	0.424 ± 0.060	0.341 ± 0.020
	DDT	0.279 ± 0.044	0.278 ± 0.012*
	Endosulfan	0.172 ± 0.021*	0.307 ± 0.013
Cytochrome $a+a_3$	None	0.322 ± 0.095	0.354 ± 0.016
	DDT	0.594 ± 0.041*	0.386 ± 0.045
	Endosulfan	0.327 ± 0.047	0.214 ± 0.007*

Values are mean ± SE from six samples in each group.

* Significantly different from controls, $p < 0.05$.

** nmole/mg protein.

activity. Results show that in lung, DDT and endosulfan though slightly different in chemical nature do not affect electron transport chain at NADH level, where as in liver both these insecticides reduce the activity of NADH oxidase and thus possibly make more NADH available for the generation of ATP. Cytochrome b,c and $a+a_3$ are electron carriers in mitochondrial electron transport chain. The reduction brought about by endosulfan in the levels of cytochrome b and c suggests that this insecticide interferes at these stages of electron transport. DDT did not affect mitochondrial cytochromes of electron transport chain. These results also show that the effects of DDT and endosulfan are different on lung electron transport chain components. In liver also endosulfan affects the levels of cytochrome b and cytochrome $a+a_3$, thereby causing an interference in a normal flow of electrons and hence in generation of ATP. DDT also affects the flow of electrons at cytochrome c level and this would possibly affect the generation of ATP. Like lung, the effects of endosulfan and DDT on liver mitochondrial electron transport components are different.

The reduction of respiratory cytochromes may cause an impairement in energy production as the transfer of electrons from substrate to O_2 (through reducing equivalents) will be minimized. These results thus indicate that intratracheally administered DDT and endosulfan certainly cause reduction in cytochrome levels and hence the energy production. At present the mechanism of reduction is not clear.

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REFERENCES

Byczkowski JZ (1973) The effect of p,p'-DDT(Dicophane) and its main metabolites on the respiration of rat liver mitochondria. Arch Toxicol 31:137-145.

Byczkowski JZ (1977) Biochemical and ultrastructural changes in biological membranes induced by p,p'-DDT (Chlorophenotanum) and its metabolites. Pol J Pharmacol Pharm 29:411-417.

Byczkowski JZ, Tuczkiewicz J (1978) comparative study of respiratory chain inhibition by DDT and DDE in mammalian and plant mitochondria. Bull Environ Contam Toxicol 20:505-512.

Cutkomp LK, Yap HH, Vea EV, Koch RB (1971) Inhibition of oligomycin sensitive (mitochondrial)Mg^{2+}ATPase by DDT and selected analyogs in fish and insect tissue. Life Sci 10 : 1201-1209.

Ela R, Chefurka W, Robinson JR (1970) In vivo glucose metabolism in the normal and poisoned cockroach, Periplanata americana. J Insect Physiol 16:2137-2156.

Gazzotti P, Malmstrom K, Crompton M (1980) In membrane Biochemistry, ed. Carafoli E and Somenza G. pp 62-76. Springer Verlag, Berlin.
Gregg CT, Johnson JC, Heisler CR, Remmert LF (1964) Inhibition of oxidative phosphorylation and related reactions in insect mitochondria. Biochim Biophys Acta 82:343-349.
Gupta PK (1976)Endosulfan-induced neurotoxicity in rats and mice. Bull Environ Contam Toxicol 15:708-713.
Gupta PK, Gupta RC (1979) Pharmacology, toxicology and degradation of endosulfan. Toxicology 13 : 115-130.
Hinkle PC, McCarty RE (1978) How cells make ATP. Scient Amer 238:104-123.
Hoeck JB, Brenster L (1974) In Alcohol and Aldehyde metabolizing systems. ed. RG Thurman, T yonetani, JR williamson and R chance. pp 351. Academic Press. New York.
Holt PF (1980) Dust elimination from pulmonary alveoli. Environ Res 23 : 224-227.
Khan HM, Cutkomp LK (1982) Effects of DDT, DDE and PCBs on mitochondrial respiration. Bull Environ contam Toxicol 29 : 577-585.
Koch RB, Cutkomp LK, Yap HH (1971) Inhibition of oligomycin sensitive and insensitive fish adenosine triphosphatase activity by chlorinated hydrocarbon insecticides. Biochem Pharmacol 20 : 3242-3245.
Lowry OH, Rosebrough NJ, Farr AL, Randall AJ (1951) Protein measurement with Folin Phenol reagent. J Biol chem 193:265-275.
Mackler B (1967) DPNH-oxidase of heart muscle. Meth Enzymol 10:261-263.
Pardini RS, Heidker JC, Baker TA, Payne B (1980)Toxicology of various pesticides and their decomposition products on mitochondrial electron transport. Arch Environ Contam Toxicol 9:87-97.
Peterson JE, Robinson WH (1964) metabolic products of p,p'-DDT in the rat. Toxicol Appl Pharmacol 6:321-327.
Rieske JS (1967)The quantitative determination of mitochondrial hemoproteins. Meth Enzymol 10:488-493.
Ritter CL, Malejka-Gignati D (1982) Mixed function oxidase in the mammary gland and liver microsomes of lactating rats. Biochem Pharmacol 31:239-247.
Waddill VH, Keeley LL (1971) Effect of DDT on biochemical functions of fat body and muscle mitochondria isolated from the cockroach, Blabera discoidalis. Pestic Biochem Physiol 1:453-457.
World Health organization Geneva (1979) DDT and its derivatives. Environmental Health Criteria 9:49-55.

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