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Effects of DDT, DDE, and PCBs on Mitochondrial Respiration[1]

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This research team has shown that DDT and certain analogs are potent inhibitors of oligomycin-sensitive, Mg^{2+}-dependent mitochondrial ATPase (O.S.-ATPase) regardless of the animal species and tissue sources examined (KOCH 1969; KOCH et al. 1971, 1972; CUTKOMP et al. 1971, 1976, 1980a,b; DESAIAH et al. 1974; CHENG & CUTKOMP 1977; SUDERSHAN & CUTKOMP 1977; KHAN & CUTKOMP 1982). O.S.-ATPase catalyzes the synthesis of ATP when it is coupled to electron flow (PENEFSKY et al. 1960; RACKER 1976) in what is described as oxidative phosphorylation. Thus, the sensitivity of this enzyme would be expected to extend to effects on oxidative phosphorylation. Two different effects have been described in the literature. PARKER (1960), BYCZKOWSKI (1973, 1977) and BYCZKOWSKI et al. (1978) reported stimulation of ATPase activity in rat liver mitochondria and thus classified DDT as an uncoupler of oxidative phosphorylation. However, all the reports dealing with insect mitochondrial preparations showed inhibition of oxidative phosphorylation (SACKLIN et al. 1955; GONDA et al. 1957; GREGG et al. 1964; ELA et al. 1970; WADDIL & KEELY 1971; CHENG 1975). PARDINI et al. (1980), in addition, found that DDT and certain related compounds inhibit the mitochondrial electron transport chain. This would be expected to secondarily reduce mitochondrial respiration and oxidative phosphorylation.

In view of the above discrepancies concerning uncoupling agents and the primary or secondary role of mitochondrial respiration, two questions need clarification: (1) Whether DDT and related compounds act as inhibitors or uncouplers of oxidative phosphorylation and (2) If the effect of these compounds on oxidative phosphorylation is primary or secondary to the inhibition of mitochondrial electron transport chain.

Data dealing with the action of DDT, its metabolite DDE and other related chlorinated compounds such as polychlorinated biphenyls (PCBs) are presented in this study.

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MATERIALS AND METHODS

Mitochondria were prepared from the red coxal muscle of adult male cockroaches, Periplaneta americana, according to the procedure of CARAFOLI et al. (1971), as modified by CHENG & CUTKOMP (1977).

The protein concentration in the final suspension of mitochondrial pellet was 0.6 to 0.8 mg/mL as determined by the method of LOWRY et al. (1951). Bovine serum albumin (BSA) was used as standard.

Oxygen consumption was determined polarographically at 27°C using Clark-type oxygen electrode (Yellow Spring Instruments Co.) and a multipen Rikadenki recorder (Rikadenki Koggo Co., Ltd., Tokyo, Japan). Oxygen concentration in the reaction mixture was calibrated by the method of ROBINSON & COOPER (1970).

The state 4 (resting) and state 3 (active) respiratory rate were determined according to the method of CHANCE & WILLIAMS (1956). The reaction mixture contained: 0.25 M sucrose, 20 mM potassium phosphate, 5 mM $MgCl_2$, 10 mM (DL)-α-glycerophosphate, and 0.5 mL cockroach muscle mitochondrial suspension (0.23-0.32 mg protein). Repeated additions of ADP produced a sequence of state 3-state 4-state 3 transitions. Respiratory control index (R.C.I.) -- state 3 rate/state 4 rate -- was calculated from these data.

Stock solutions of all chemicals (DDT, DDE, Aroclor 1242, and Aroclor 1254) were prepared in ethanol. Stock solutions were delivered by a Hamilton microsyringe in 1-2 μL volumes into rapidly stirring reaction mixtures to achieve the desired final concentrations as shown in Table 1. Ethanol alone was added to the control determinations.

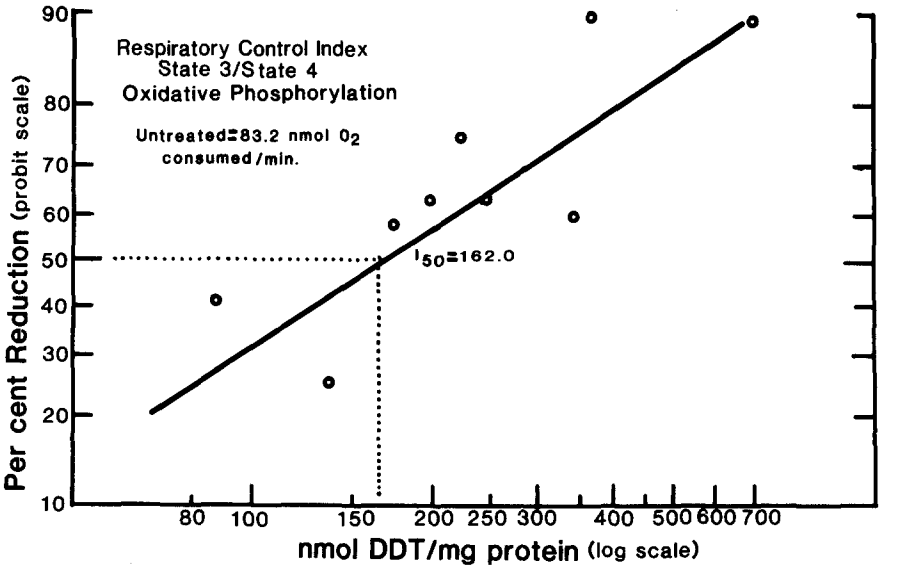
The concentration-response data of the R.C.I. were analyzed following the probit program of DAUM (1970) and FINNEY (1971).

RESULTS

Cockroach muscle mitochondria showed satisfactory resting (state 4) and active (state 3) respiratory rates in the presence of the substrate (DL)-α-glycerophosphate (Figs. 2 and 3). The ADP/O ratio varied between 1.95 and 2.1 (theoretical value is 2.0).

Effects of DDT in Mitochondrial Respiration

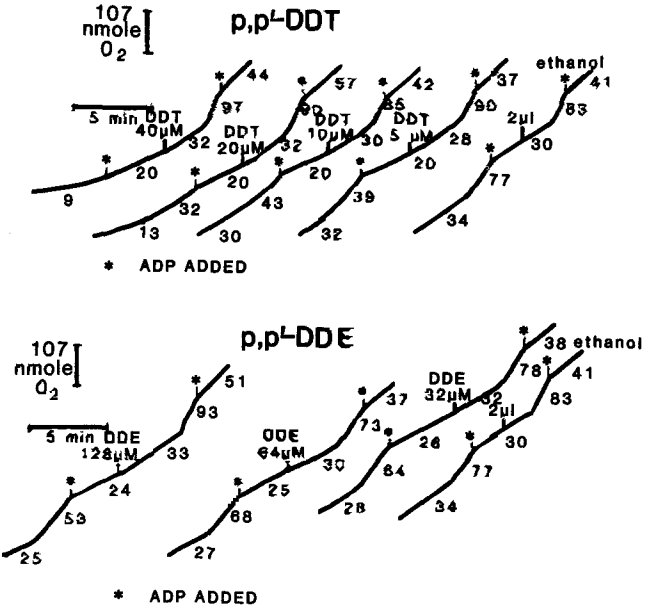
The effect of DDT on state 3 respiration was more pronounced and concentration-dependent that on state 4 respiration (Fig. 1 and 2 and Table 1). The addition of ADP caused an increasingly weaker stimulation of O_2 consumption in the presence of 87 to 368 nmol DDT/mg protein. At 696 nmol DDT/mg protein, however, addition to ADP failed to elicit a transition to 3 indicating a total inhibition of oxidative phosphorylation. Furthermore,



F 1. Dosage-response curve of DDT based upon 9 concentrations of DDT as it reduces (or inhibits) oxidative phosphorylation as measured by the Respiratory Control Index (R.C.I.) determined at 27°C.

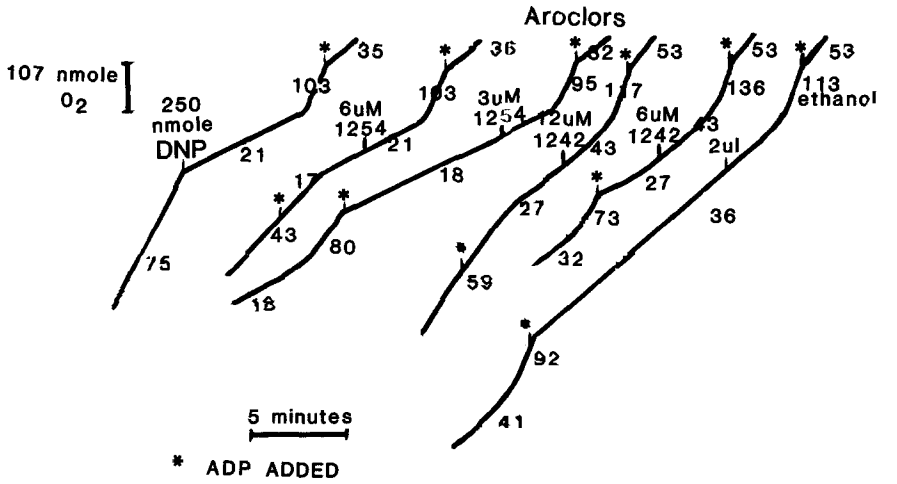
Table 1. Effects of DDT on state 4 and state 3 Respiration and Respiratory Control Index (R.C.I.) of Cockroach Coxal Muscle Mitochondria. Statistical treatment of DDT responses (R.C.I.) presented in detail in Figure 1. DDE response less than 50% thus was not amenable to analysis.

	nmol O_2 consumed/min			
	Insecticide: nmol/mg protein	State 4	State 3 220 nmol ADP	R.C.I. (State 3/State 4)
DDT	0	--	83.2	2.70
	87	20.0	39.4	1.95
	174	20.3	42.8	2.10
	245	22.5	36.0	1.60
	348	20.3	33.8	1.67
	696	20.3	9.0	0.44
DDE	392	26.0	64.2	2.47
	784	25.0	67.2	2.70
	1568	24.0	53.0	2.21



F 2. Examples of the effects of DDT and DDE on cockroach coxal muscle mitochondrial respiration and oxidative phosphorylation. The oxygen electrode traces obtained with 10 mM (DL)-α-glycerophosphate and 0.23-0.32 mg mitochondrial protein in 4 mL reaction mixture at 27°C. Where indicated, 220 nmoles of ADP were added in 5 μL volume.

DDT and DDE added in 1-2 μL ethanol solution. Numbers indicated rate of oxygen consumption in nmoles/min.



F 3. Comparison of the effects of PCBs (Aroclor 1242 and 1254) and DNP on cockroach coxal muscle mitochondrial respiration. Reaction conditions as in Figure 2. The numbers indicate rate of oxygen consumption in nmoles/min.

addition of ADP in this case caused a further decline in state 4 or resting respiratory rate indicating that the mitochondria may have been damaged (Fig. 2).

Probit analysis of the concentration-dependent decline in R.C.I. yielded an IC_{50} (concentration causing 50% inhibition) value of 162 nmol DDT per mg protein (Fig. 1). Reductions in the resting (state 4) respiration were obvious immediately following the addition of DDT to the reaction mixture (Fig. 2). Inhibition, however, was not concentration dependent and ranged between 28 to 42% at 87 to 696 nmol DDT per mg protein (Table 1).

Effects of DDE on Mitochondrial Respiration

DDE at 1.5 µmole per mg protein (9 times the IC_{50} concentration of DDT) reduced the state 3 respiration and R.C.I. by only 36% (Table 1 and Figure 2). Similarly, state 4 respiration was much less sensitive to DDE than DDT, giving 17 to 27% reduction at 0.39 to 1.57 µmole/mg protein.

Effects of PCBs on Mitochondrial Respiration

The effects of PCBs were related to both the concentration and the degree of chlorination of the compounds. No effects were obvious below a critical concentration of these compounds. Thus, Aroclor 1242 (42% chlorination) at 12 µM uncoupled the respiration (Fig. 3). The uncoupling effects continued at all concentrations tested above these critical levels.

In contrast to an instantaneous uncoupling effect produced by 2,4-dinitrophenol (DNP at 250 nmole) there was about a 2-3 min lag period before uncoupling effect of the PCBs became obvious

DISCUSSION

Studies using mitochondrial preparations have shown that the inhibition of state 3 respiration is an indication of energy transfer inhibition while the inhibition of state 4 shows an inhibition of electron transport (ERNSTER et al. 1966; OZAWA et al. 1971; YONEDA 1967). DDT and DDE inhibited both of these processes in cockroach coxal muscle mitochondria. Oxidative phosphorylation (state 3) was inhibited and the dose-response (Fig. 1) showed sensitivity to DDT (an IC_{50} of 162 nmoles based upon R.C.I.) and DDE than the electron transport (state 4) (Table 1). This observation parallels earlier reports of SACKLIN et al. (1955), GONDA et al. (1957, GREGG et al. (1964), and WADDIL & KEELEY (1971). Greater efficacy of DDT than DDE correlates with the action of these compounds on O.S.-ATPase as reported by CHENG & CUTKOMP (1977). This contrasts with nearly equal inhibitory effects from DDT and DDE on respiration of rat liver mitochondria reported by BYCZKOWSKI et al. (1978). They report that 120 and 180 nmoles of insecticide/mg of mitochondrial protein is required to achieve 50% inhibition. The present results do not rule out the possibility that reductions in state 3 respiratory rate may, in part, reflect the reduced rates of electron transport.

BYCZKOWSKI et al. (1978) point out that R.C.I. is an indicator of mitochondrial integrity rather than oxidative phosphorylation efficiency. Since the effects of DDT and DDE on state 3 in the present study were found to be concentration dependent, it is unlikely that any damage to the mitochondrial membranes occurred. However, damage could have occurred at 696 nmole DDT/mg protein (high concentration) as pointed out earlier. Indeed BYCZKOWSKI (1977) showed damage to rat liver mitochondrial membranes in electron micrographs using 500 nmol DDT and DDE per mg protein. A comparison with occupationally exposed adults RADOMSKI et al. (1971) would indicate that individuals could have about 20 nmol DDT/mg protein, assuming about 16% protein in whole blood.

Clearly, the present results show that DDT and DDE do not stimulate mitochondrial oxygen consumption in contrast to DNP, the classical uncoupler. The present data, in accord with earlier reports from this laboratory in which these compounds inhibited rather than stimulated O.S.-ATPase, demonstrate that DDT and DDE are inhibitors of oxidative phosphorylation rather than uncouplers. The compounds are thus acting at the final step in the respiratory chain and have a distinctly different action than rotenone which blocks oxidation of $NADH_2$, an earlier event in carbohydrate metabolism. An additional compound, ruthenium red, does not prevent the phosphorylation of ADP, but abolishes calcium stimulated respiration.

Extreme sensitivity of oxidative phosphorylation to DDT can be helpful in explaining certain physiological responses of an organism to DDT exposure. For example, MAJNO et al. (1960) stated, using experimental evidence 'as soon as the oxygen supply is curtailed, the cellular "sodium pump"-lacking energy-becomes ineffective and sodium ions begin to diffuse in, while potassium leaks out.' This effect would impair nerve conduction in animals. In addition to the critical effect which could indirectly affect nerve impulses the limitation of ATP synthesis and available energy can have other adverse effects. One which we have studied involves a decline in the ability to transport calcium in avian shell gland. This effect has recently been proposed as the basis for DDE-induced avian eggshell thinning (KHAN & CUTKOMP 1982).

Uncoupling effects on oxidative phosphorylation by PCBs in cockroach coxal muscle mitochondria are in agreement with those of SIVALINGAN et al. (1973), on rat liver mitochondria. At a rather high concentration of 0.33 mM PCBs, PARDINI (1971) showed them to be inhibitors of electron transport. That the effects may vary with mitochondrial source and concentration employed is obvious from the studies of DESAIAH et al. (1972), CUTKOMP et al. (1972), and KOCH et al. (1972). These authors found that Arcolors 1242 and 1254 stimulated the O.S.-ATPase activities from fish kidney and muscle at lower concentrations. Identical concentrations tested on fish brain O.S.-ATPase resulted in inhibition and gave no stimulation.

In summary, both DDT and DDE effectively reduce oxidative phosphorylation as determined from cockroach muscle mitochondria. DDT is more effective as was also determined for inhibition of oligomycin-sensitive Mg^{2+}ATPase. The PCBs tested were uncouplers of oxidative phosphorylation.

REFERENCES

BYCZKOWSKI, J. Z.: Arch. Toxicol. 31,137 (1973).

_____: Pol. J. Pharmacol. Pharm. 29,411 (1977).

BYCZKOWSKI, J. Z., and J. TUCZKIEWICZ: Bull. Environ. Contam. Toxicol. 20,505 (1978).

CARAFOLI, E., R. G. HANSFORD, G. SACKTON, and A. L. LEHNINGER: J. Biol. Chem. 246,964 (1971).

CHANCE, B., and G. R. WILLIAMS: Adv. Enzymol. 17,65 (1956).

CHENG, E. Y.: The inhibitory effects of DDT and its analogues on the biochemical aspects of ATPases in the American cockroach, Periplaneta americana (L). Ph.D. Dissertation, University of Minnesota, St. Paul, Minn. (1975).

CHENG, E. Y., and L. K. CUTKOMP: Pestic. Biochem. Physiol. 7,360 (1977).

CUTKOMP, L. K., D. DESAIAH, E. Y. CHENG, E. V. VEA, and R. B. KOCH: Pestic. Biochem. Physiol. 6,203 (1976).

CUTKOMP, L. K., H. H. YAP, D. DESAIAH, and R. B. KOCH: Environ. Health Perspect. 1,165 (1972).

CUTKOMP, L. K., H. H. YAP, E. V. VEA, and R. B. KOCH: Life Sci. Pt. II 10,1201 (1971).

DAUM, R. J.: Bull. Entomol. Soc. Amer. 16,10 (1970).

DESAIAH, D., L. K. CUTKOMP, and R. B. KOCH: Pestic. Biochem. Physiol. 4,232 (1974).

DESAIAH, D., L. K. CUTKOMP, H. H. YAP, and R. B. KOCH: Biochem. Pharmacol. 21,857 (1972).

ELA, R., W. CHEFURKA, and J. R. ROBINSON: J. Insect Physiol. 16,2137 (1970).

ERNSTER, L., C.-P. LEE, and S. JANDA: The reaction sequence in oxidative phosphorylation. In "Biochemistry of Mitochondria' (E. C. Slater, Z. Kaniuga and Wojtczak, eds.). Acad. Press, N.Y. (1966).

FINNEY, D. J.: Probit analysis. 3 ed. Cambridge Univ. Press, London (1971).

GONDA, O., A. KLAUSZNER, and Y. AVI-DOR: Biochem. J. 73,583 (1959).

GREGG, C. T., J. C. JOHNSON, C. R. HEISLER, and L. F. REMMERT: Biochem. Biophys. Acta 82,343 (1964).

KHAN, H. M., and L. K. CUTKOMP: Arch. Environ. Contam. Toxicol. (In Press) (1982).

KOCH, R. B.: J. Neurochem. 16,269 (1969).

KOCH, R. B., L. K. CUTKOMP, and H. H. YAP: Biochem. Pharmacol. 20,3243 (1971).

KOCH, R. B., D. DESAIAH, H. H. YAP, and L. K. CUTKOMP: Bull. Environ. Contam. Toxicol. 7,87 (1972).

LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR, and R. J. RANDALL: J. Biol. Chem. 193,265 (1951).

MAJNO, G., M. LAGATTUTA, and T. E. THOMPSON: Virchow Arch. Path. Anat. 333,421 (1960).

OZAWA, T., J. ASAI, and K. UTSUMI: Oxidative phosphorylation and respiratory patterns of mitochondria. In 'Mitochondria-Molecular organization and Physiological Chemistry' (T. Ozawa, J. Asai and K. Utsumi, eds.). Nankado Press, Tokyo (1971).

Pardini, R. S.: Bull. Environ. Contam. Toxicol. 6,539 (1971).

PARDINI, R. S., J. C. HEIDKER, T. A. BAKER, and B. PAYNE: Arch. Environ. Contam. Toxicol. 9,87 (1980).

PARKER, V. H.: Biochem. J. 77,74 (1960).

PENEFSKY, H. S., M. E. PULLMAN, A. DATTA, and E. RACKER: J. Biol. Chem. 235,3330 (1960).

RACKER, E.: A new look at mechanisms in bioenergetics. Academic Press, New York, pp. 67-87 (1976).

RADOMSKI, J. L., E. ASTOLFI, W. B. DEICHMANN, and A. A. REY: Toxicol. Appl. Pharm. 20,186 (1971).

ROBINSON, J., and J. M. COOPER: Anal. Biochem. 33,390 (1970).

SACKLIN, J. A., L. C. TERRIERE, and L. F. REMMERT: Science 122, 377 (1955).

SIVALINGAN, P. M., T. YOSHIDA, and Y. INADA: Bull. Environ. Contam. Toxicol. 10,242 (1973).

SUDERSHAN, P., and L. K. CUTKOMP: Life Sci. 21,921 (1971).

WADDIL, V. H., and L. L. KEELEY. Pestic. Biochem. Physiol. 1, 453 (1971).

YONEDA, M.: Oxidative phosphorylation of mitochondria. In 'Biological Membrane, Experimental Techniques' (T. Oonishi, ed.). Nankodo Press, Tokyo (1967).

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