Biochimica et Biophysica Acta, 1060 (1991) 166–174

ADONIS 000527289100232O

BBABIO 43484

Mitochondrial bioenergetics as affected by DDT

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(Received 28 January 1991)

Key words: Pesticide; Mitochondrial membrane potential; Phosphorylation; (DDT)

The organochloride insecticide DDT (2,2-bis(*p*-chlorophenyl)-1,1-trichloroethane) depresses the phosphorylation efficiency of mitochondria as inferred from the decrease of respiratory control ratio (RCR) and *P/O* ratio, perturbations of transmembrane potential ($\Delta\Psi$) fluctuations associated with mitochondrial energization and phosphorylative cycle induced by ADP. DDT depresses the $\Delta\Psi$ developed by energized mitochondria and prevents complete repolarization, that is delayed and resumed at a lower rate. The inhibitory action of DDT on phosphorylation efficiency may result from: (1) a direct effect on the ubiquinol-cytochrome *c* segment of the redox chain; (2) direct action on the ATP-synthetase complex; (3) partial inhibition of the phosphate transporter. DDT preferentially interacts with phosphorylation process in relation to respiration. High concentrations of DDT induce destruction of the structural integrity of mitochondria.

Introduction

DDT is an organochloride pesticide widely used in the control of insect pests and vector-borne diseases (e.g., malaria, typhus) [1] with a major impact on modern society. In spite of considerable benefits to agriculture and public health, the high persistance and other risks led to banishment of DDT from agriculture. However, it is still in use in countries of third world for the control of disease-carrying vectors.

The precise biochemical mechanisms responsible for DDT toxicity are poorly understood. The toxicity has been assigned mainly to effects on the nervous system by poisoning the axon membranes [2]. This effect has been correlated with modification of the Na^+ and K^+ conductance of the axon membrane [3]. The pesticide has irreversible effects on impulse conduction in nerve cells, producing loss of coordination followed by tremors, convulsion, paralysis and death [2].

The high lipophilicity of DDT explains the broad basic membrane mechanisms disturbed by the pesticide [4–9]. DDT affects the permeability of liposomes and native membranes to K^+ [9] as well as to other electrolytes and non-electrolytes [7,8]. Additionally, DDT alters the thermotropic behaviour of lipid bilayers [10–12]. Furthermore, DDT affects membrane ATPases associated with transport and energy transduction [4,5,8,13]. The observed effects at the membrane level may additionally be responsible for toxic actions of DDT in vivo.

DDT has a low immediate toxicity to mammals (LD_{50} for rats = 250 mg/kg [14]), including man. However, the possible long-term effects resulting from frequent contact with the compound for long periods of time remain to be elucidated. As a consequence of the high stability, decades of exposure will occur after the use of DDT has been discontinued.

Early reports have implicated DDT in dysfunctions of mitochondrial processes regardless of the species and tissue examined [13,15–19]. The limitation of ATP synthesis was suggested to be implicated in the toxicology of the pesticide. However, data concerning the effects either on mitochondrial ATPase or on oxidative redox chain are often in conflict and no clear description has been offered.

The present study has been designed to investigate the effects of the pesticide on redox complexes, membrane energization and phosphorylation-related processes.

Material and Methods

Isolation of mitochondria. Mitochondria were isolated from liver of Sprague-Dawley rats (200–300 g) of either sex by conventional methods [20] with slight

Abbreviation: DDT, 2,2-bis(*p*-chorolphenyl)-1,1-trichloroethane.

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modifications. Homogenization medium contained 0.25 M sucrose, 5 mM Hepes (pH 7.4), 0.2 mM EGTA, 0.1 mM EDTA and 0.1% defatted bovine serum albumin. EGTA, EDTA and bovine serum albumin were omitted from the final washing medium, adjusted at pH 7.2. Protein was determined by the biuret method calibrated with bovine serum albumin [21].

Mitochondrial respiration. Oxygen consumption of isolated mitochondria was monitored polarographically with a Clark oxygen electrode connected to a suitable recorder in a 1 ml thermostated water-jacketed closed chamber with magnetic stirring, at 25 °C. The standard respiratory medium consisted of 130 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 5 mM KH_2PO_4, 5 mM Hepes (pH 7.2) and 2 μM rotenone, when succinate was used as substrate. DDT was added in ethanolic solutions (up to 2 μl) to the reaction medium with mitochondria (1 mg) and allowed to incubate for 6 min before the addition of succinate or the beginning of other reactions. This time was chosen because of time-dependent DDT effects which were maximal at 6 min. The respiratory control ratio (RCR) and P/O ratios were calculated according to Chance and Williams [22].

Membrane potential ($\Delta\Psi$) measurements. A sensitive electrode of tetraphenylphosphonium prepared according to Kamo et al. [23] was used to determine the $\Delta\Psi$ from the distribution of TPP^+ across the mitochondrial membrane. The voltage response of the TPP^+ electrode to log [TPP^+] was linear with a slope of 59 ± 1, in good agreement with the Nernst equation. Calibration runs in the presence of DDT excluded any direct interference of the pesticide on electrode signal. Reactions were carried out in an open vessel maintained at 25 °C, with efficient magnetic stirring, in 1 ml of the standard respiratory medium supplemented with 8 μM TPP^+, i.e., low enough to avoid possible toxic effects on mitochondria [24,25]. The $\Delta\Psi$ was estimated as indicated by Kamo et al. [23] from the following equation (at 25 °C):

$$\Delta\Psi(\text{mV}) = 59 \log(v/V) - 59 \log(10^{\Delta E/59} - 1)$$

where v, V and ΔE are mitochondrial volume, volume of incubation medium and deflection of the electrode potential from the baseline, respectively. A matrix volume of 1.1 μl per mg protein was assumed. No correction was made for the 'passive' binding of TPP^+ to the mitochondrial membranes, since the purpose of the experiments was to show relative changes in potentials rather than absolute values. As consequence, we can anticipate some overestimation for $\Delta\Psi$ values. Effects of DDT on passive TPP^+ binding measured under uncoupling conditions (FCCP) were negligible.

Enzymatic activities. Succinate dehydrogenase activity was measured polarographically at 25 °C in 1 ml of the standard reaction medium supplemented with 5 mM succinate, 2 μM rotenone, 0.1 μg antimycin A, 1 mM KCN, 0.3 mg Triton X-100 and 0.5 mg mitochondria. The reaction was initiated by the addition of 1 mM PMS (phenazine methosulfate). Initial rates of the activity were calculated to avoid the problems of O_2 starvation and product accumulation.

Cytochrome oxidase activity was measured polarographically at 25 °C in 1 ml of the standard reaction medium supplemented with 2 μM rotenone, 10 μM cytochrome *c*, 0.3 mg Triton X-100 and 0.5 mg mitochondria. The reaction was initiated by the addition of 5 mM ascorbate plus 0.25 mM TMPD (*N*, *N*, *N'*, *N'*-tetramethyl-*p*-phenylenediamine).

Succinate-cytochrome *c* reductase activity was measured spectrophotometrically at 25 °C by following the reduction of oxidized cytochrome *c* as an increase in absorbance at 550 nm. The reaction was initiated by the addition of 5 mM succinate to 2.5 ml of the standard reaction medium supplemented with 2 μM rotenone, 1 mM KCN, 54 μM cytochrome *c* and 0.6 mg mitochondria. The reductase measurements account only for mitochondria having a broken outer membrane, since cytochrome cannot permeate across the intact membrane. However, DDT disrupting effects can be ruled out, since instead of stimulation, the activity is actually depressed by the insecticide (see Results).

ATP synthesis was determined by measuring the changes in pH of the medium associated with ATP synthesis as reported by Nishimura et al. [26], at 25 °C, in 2 ml of medium consisting of 130 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 2 mM KH_2PO_4, 2 μM rotenone, 5 mM succinate, at pH 7.2. The insecticide was injected into the medium after the addition of mitochondria (1 mg) and allowed to incubate for 6 min. The reaction was started by the addition of 150 μM ADP.

ATPase activity was determined by monitoring the pH change associated with ATP hydrolysis. The reaction was carried out in 2 ml of the respiratory medium lightly buffered with 0.5 mM Hepes (at pH 7.2), and supplemented with 2 μM rotenone and mitochondria (1 mg). The reaction was initiated by the addition of 3 mM Mg-ATP. The addition of oligomycin (2 μg) at the end of the assays completely abolished H^+ productions. H^+ production was calculated after an elapsed time of 3 min from the start of the reaction.

Measurement of Ca uptake. Ca^{2+} uptake was followed by using a Ca-selective electrode of the neutral carrier type according to Madeira [27]. Mitochondria (0.3 mg for succinate or 0.2 mg for ATP) in 1 ml of the standard respiratory medium without phosphate and supplement with 50 μM $CaCl_2$ and 2 μM rotenone (for succinate) or 2 μM rotenone, 1 mM KCN and 50 μM $CaCl_2$ (for ATP) were energized with 2.5 mM succinate or 2 mM ATP-Mg after 6 min of incubation.

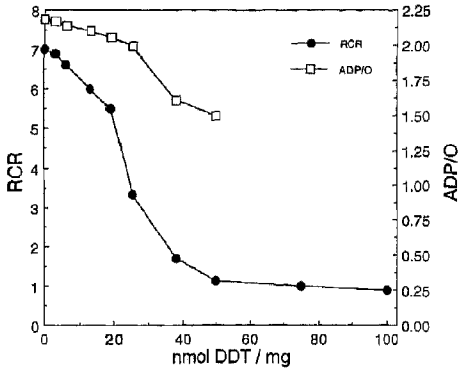


Fig. 1. Effect of DDT on mitochondrial respiratory indexes (RCR and *P/O*). Mitochondria (1 mg) incubated in 1 ml of the respiratory medium for 6 min prior addition of succinate (state 4) were supplemented with ADP (0.12 mM) to induce state 3 condition.

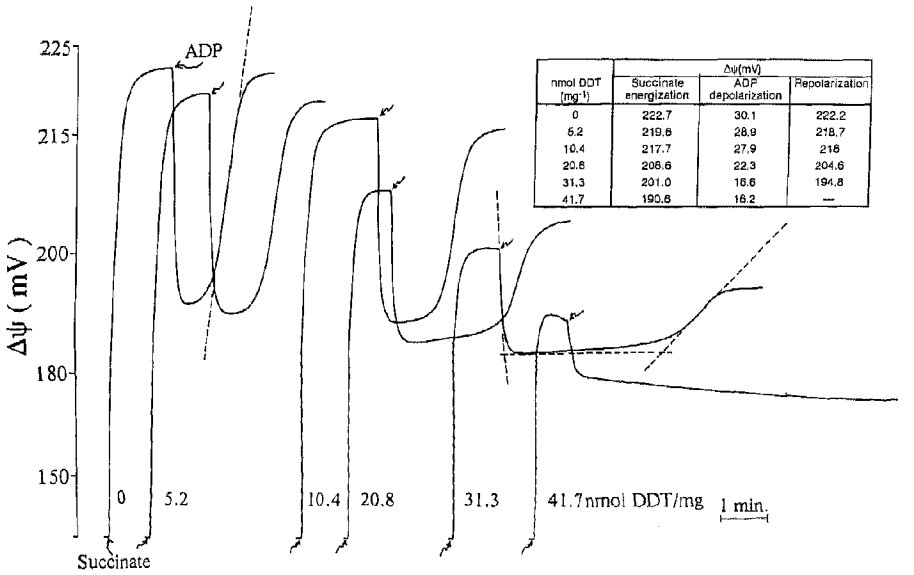
Calibration was carried out by adding known amounts of $CaCl_2$ before and at the end of the assays. Ca^{2+} taken up by mitochondria was calculated after 3 min from the energization. The relatively high Ca^{2+} concentration (50 μM) was chosen to permit reliable uptake measurements with the Ca^{2+} electrode. Since the total amount of Ca^{2+} taken up is relatively limited, the external Ca^{2+} concentration sensed by the electrode decreases by a small amount, thus allowing an almost linear response of the electrode.

Mitochondrial swelling. Mitochondrial osmotic volume changes were measured by the apparent absorbance changes at 540 nm with a suitable spectrophotometer-recorder set-up. The reactions were carried out at 25 °C in 3 ml of the required isoosmotic media as indicated in the legends to figures.

Chemicals. All chemicals were of analytical grade for research. DDT (99.9% pure), was obtained from Ciba-Geigy, and dissolved in absolute ethanol. Equal volumes of ethanol solvent added to controls (up to 2 μl) were without effect on measured activities.

Results

Fig. 1 summarizes the effect of DDT on respiratory control ratio (RCR) and phosphorylative index (*P/O* ratio) of liver mitochondria using succinate as respiratory substrate. Both indexes are decreased, suggesting a depressive effect on the phosphorylation capacity. The *P/O* ratios were measured only up to 50 nmol DDT/mg protein, since for higher proportions RCR approaches 1.0, i.e., ADP does not induce stimulation of respiratory rate. For proportions of DDT higher than 60 nmol DDT/mg, the addition of ADP induces inhibition of mitochondrial respiration, suggesting



nmol DDT (mg·l)	Δψ(mV) Succinate energization	ADP depolarization	Repolarization
0	222.7	30.1	222.2
5.2	219.6	28.9	218.7
10.4	217.7	27.9	216
20.8	206.6	22.3	204.6
31.3	201.0	16.6	194.8
41.7	190.6	16.2	—

Fig. 2. Effect of DDT on the mitochondrial transmembrane potential ($\Delta\Psi$). Mitochondria (0.6 mg) in 1 ml of the standard respiratory medium supplemented with 8 μM TPP^+ were energized with 5 mM succinate after incubation; addition of ADP (0.12 mM) induces state 3 condition. The amounts of DDT are indicated on traces. Note that DDT depresses the total developed $\Delta\Psi$, the depolarization induced by ADP and lengthens the lag phase preceding repolarization. Also, repolarization resumes at a reduced rate and level. The traces represent typical recordings from several experiments with different mitochondrial preparations. The traces represent typical recordings from several experiments with different mitochondrial preparations. Lag phase is the period between the end of depolarization and the onset of repolarization; it was calculated after drawing tangents to the slopes (dashed lines). Repolarization rate is the slope of repolarization trace as calculated from the tangent.

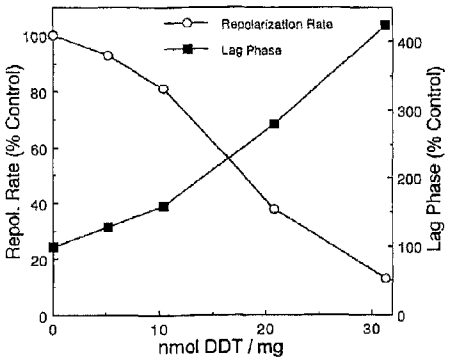


Fig. 3. Effects of DDT on the lag phase preceding repolarization and on the rate of repolarization. Experimental conditions were as described for Fig. 2.

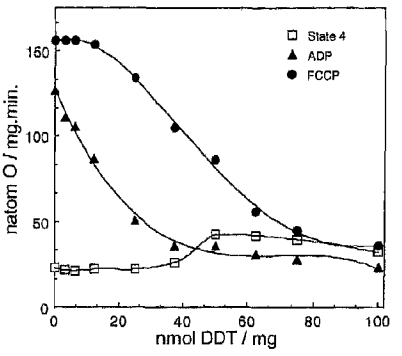


Fig. 4. Effect of DDT on respiratory rates of mitochondria. Mitochondria (1 mg) were incubated in 1 ml of the respiratory standard medium. The ADP-stimulated respiration was initiated by the addition of 1.5 mM ADP. The FCCP-stimulated respiration was initiated by the addition of 1.5 μM FCCP. State 4 respiration was initiated by the addition of 5 mM succinate after 6 min of incubation. ADP or FCCP were added 2 min after the initiation of state 4 respiration.

deleterious effects further confirmed in osmotic studies. The depressive effect of DDT on the phosphorylation capacity of mitochondria was further confirmed by monitoring the transmembrane potential ($\Delta\Psi$) fluctuations associated with mitochondrial respiration and the phosphorylation cycle induced by ADP (Fig. 2). The drop of about 30 mV induced by DDT ($\approx$ 40 nmol/mg) indicates a direct effect of the insecticide on the mitochondrial redox chain, since complete inhibition of phosphorylation linked to state 4 respiration would produce a drop as much as 6 mV [28].

Upon ADP addition, the $\Delta\Psi$ abruptly decays to -193 mV, since ATP-synthetase used $\Delta\Psi$ to phosphorylate added ADP. However, in addition to the depressed level of respiratory $\Delta\Psi$, the insecticide consistently decreases the depolarization amplitude following ADP addition and lengthens the lag phase preceding repolarization. Furthermore, DDT progressively depresses the level of $\Delta\Psi$ after repolarization and the repolarization rate. This effect is described in Fig. 3 together with the lag phase preceding repolarization. For DDT/protein approaching 40 nmol/mg, repolarization recovery does not resume.

Respiratory rates characteristics of state 4 (succinate alone), FCCP-stimulated respiration, and ADP-stimulated respiration (state 3) are depicted in Fig. 4. The effect of DDT on FCCP-induced respiration reflects its interaction with the mitochondrial redox chain and confirms the impairment in the electron transfer along the respiratory chain also inferred from Fig. 2. Additionally, state 3 respiration is more sensitive to inhibition by DDT than uncoupled respiration. Therefore, in addition to the redox chain, the insecticide affects the phosphorylation process (ATP-synthetase itself, adenine nucleotide carrier and/or phosphate carrier). Furthermore, stimulation of state 4 respiration at 50 nmol DDT/mg protein was observed, suggesting uncoupling promoted by the insecticide or non-specific effects on the structural integrity of the mitochondrial membrane. Actually, 50 nmol DDT/mg protein or above promote oxidation of exogenous NADH (not shown), reflecting disruption of membrane structural integrity. Depending on the preparation, the DDT/protein ratio at which this damage is observed occurs in a narrow range of 40–50 nmol DDT/mg protein, as also inferred from reversible osmotic changes in the presence of DDT up to 50 nmol/mg protein (Fig. 5). Respiring mitochondria in the presence of valinomycin revert the swelling induced by phosphate when the respiration is inhibited with cyanide. However, above 50 nmol DDT/mg protein, the reversion of swelling is very limited, reflecting membrane instability in the

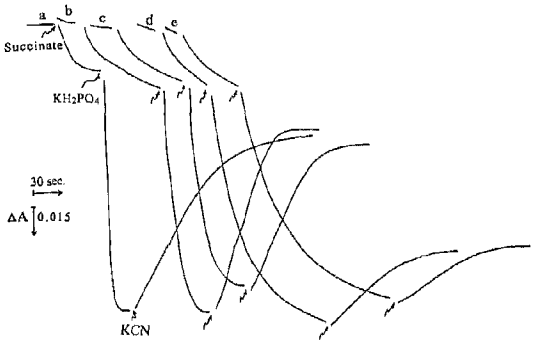


Fig. 5. Effect of DDT on osmotic volume changes of mitochondria. Mitochondria (0.5 mg) were incubated in 3 ml of the standard respiratory medium without phosphate, and supplemented with 2 μM rotenone and 0.25 μg valinomycin. Additions of 5 mM succinate, 5 mM KH_2PO_4 and 1 mM KCN were as indicated. Tracings (*a*) through (*e*) contained 0, 25, 38, 50 and 75 nmol DDT/mg protein, respectively. Note that DDT decreases the rate of swelling and shrinkage as consequence of partial inhibition of the phosphate carrier, and that the osmotic changes only are reversible for low amounts of DDT. The traces represent typical recordings.

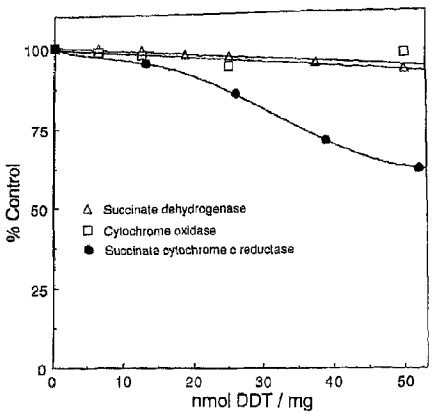


Fig. 6. Effect of DDT on cytochrome oxidase, succinate dehydrogenase, and succinate–cytochrome-*c* reductase. Experimental conditions were as described in Methods. DDT acts on the ubiquinol-cytochrome *c* segment of the redox chain.

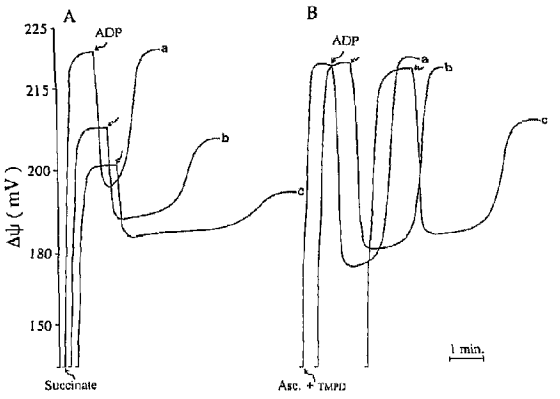


Fig. 7. Effect of DDT on the mitochondrial transmembrane electrical potential ($\Delta\Psi$). Mitochondria (0.5 mg) were energized with 5 mM succinate (A) or 5 mM ascorbate plus 0.25 mM TMPD (B). Other experimental conditions were as described for Fig. 2. Even though DDT does not significantly affect mitochondrial energization with ascorbate, it alters the normal fluctuations of $\Delta\Psi$ associated with the phosphorylation cycle. Tracings (a) through (c) were recorded in presence of 0, 25 and 38 nmol DDT/mg protein, respectively. The traces represent typical recordings.

mitochondrial population. Probably, membrane disruption has occurred after the course of swelling with consequent loss of osmotic activity, therefore, not reversed by cyanide.

The effects of DDT on the activity of mitochondrial redox components are documented in Fig. 6. Succinate dehydrogenase and cytochrome *c* oxidase are insensitive to DDT, but succinate cytochrome *c* reductase is markedly inhibited. Therefore, the effect of DDT is localized to the ubiquinol-cytochrome *c* segment of the redox chain. Transmembrane potential recordings further confirm that the terminal segment of the redox chain is DDT-insensitive, since the $\Delta\Psi$ developed upon energization with ascorbate/TMPD is not affected (Fig. 7). Even so, depolarization induced by ADP is decreased by DDT, the lag phase preceding repolarization is extended, and the repolarization recovery is only partial.

The inhibition of ATP synthesis is detailed in Fig. 8 showing the alkalinization associated to ATP synthesis. ATP synthesis rate decreases as a function of DDT concentration. Particularly interesting are the effects induced by small amounts of DDT (3–6 nmol) not effective on respiration (Fig. 4). The $\Delta\Psi$ supported by ATP in non-respiring mitochondria decreases with

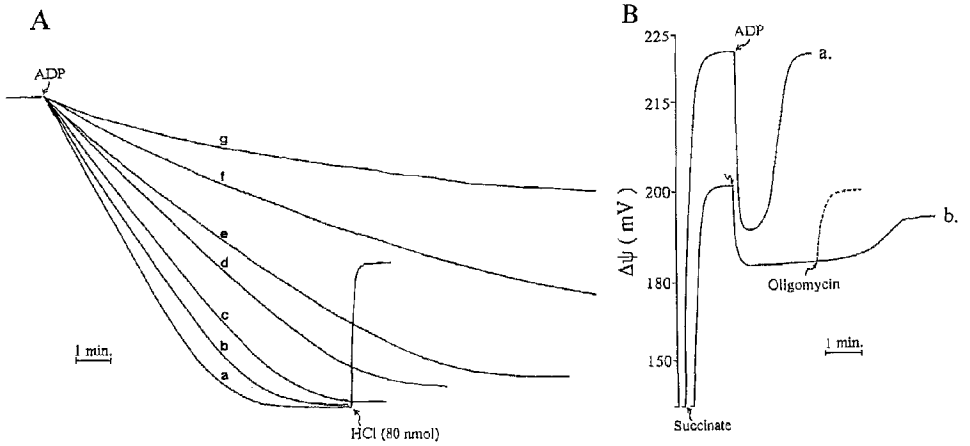


Fig. 8. (A) Effect of DDT on ΔpH changes associated with ATP synthesis. Conditions were as described in Material and Methods. Tracings a to g were recorded in the presence of 0, 3, 6, 9, 13, 16 and 19 nmol DDT/mg protein, respectively. (B) Effect of oligomycin on the mitochondrial transmembrane potential ($\Delta\Psi$). Note that the addition of oligomycin during the lag phase preceding repolarization brings about an immediate and complete recovery of $\Delta\Psi$ to state 4 level. (A) 0; (b) 19 nmol DDT/mg protein.

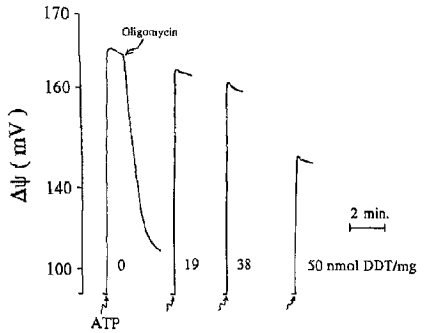


Fig. 9. Effect of DDT on the mitochondrial transmembrane potential ($\Delta\Psi$) supported by ATP energization. Mitochondria (1 mg) were incubated in the standard respiratory medium supplemented with 2 μM rotenone, 1 mM KCN and 8 μM TPP^+. After 6 min of incubation, energization was accomplished by adding 2 mM Mg-ATP. The amounts of DDT are indicated on the traces.

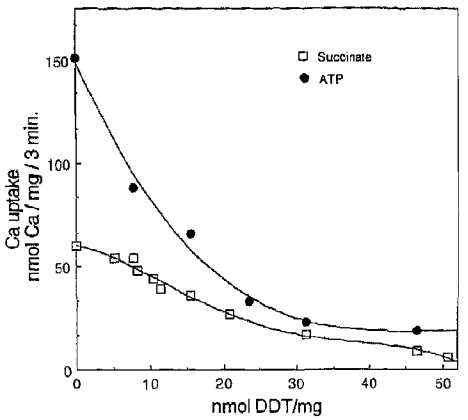


Fig. 10. Effect of DDT on Ca^{2+} uptake driven by respiration or ATP hydrolysis. Experimental conditions were as described in Material and Methods.

DDT, apparently reflecting an inhibitory action on the ATPase activity (Fig. 9). However, it must be stressed that the ATPase activity measured by detection of H^+ (Table I) clearly shows a stimulatory action of the insecticide indicating some sort of energy dissipation through the enzyme complex.

The effects of DDT on ATP-dependent and respiration-dependent Ca^{2+}-uptake were also studied (Fig. 10). The rate and amount of Ca^{2+} taken up were both depressed by the insecticide, confirming direct action on the redox chain and ATP-synthetase (ATPase).

The putative effect of DDT on the phosphate carrier was studied by monitoring the swelling of mitochondria in isoosmotic solutions of ammonium phosphate (Fig. 11). Apparently, DDT causes a partial inhibition of phosphate transport, since the rate of mitochondrial swelling is depressed when the insecticide is present. Therefore, the depression of ATP synthesis is partially related to the observed inhibition of mitochondrial phosphate transport.

The ΔpH induced by mitochondrial respiration was monitored by adding nigericin after reaching the $\Delta\Psi$ characteristic of state 4 (Fig. 12). Nigericin allows an exchange of protons for K^+, promoting the conversion of ΔpH to $\Delta\Psi$ leading to an increase of membrane potential. The extra 17 mV of $\Delta\Psi$ corresponds to a ΔpH of about 0.28. Since ΔpH is not altered, a classic uncoupling action of DDT (FCCP type) is ruled out. Only large amounts of DDT promote extensive permeability to H^+ as consequence of non-specific destruc-

TABLE I

Effect of DDT on ATPase activity of rat liver mitochondria

DDT (nmol mg^{-1})	ATPase activity (nmol H^+ min^{-1} mg^{-1})
0	7.2
6.3	7.6
9.4	12.3
18.8	27.0
25.0	30.3
37.5	37.0
50.0	47.0

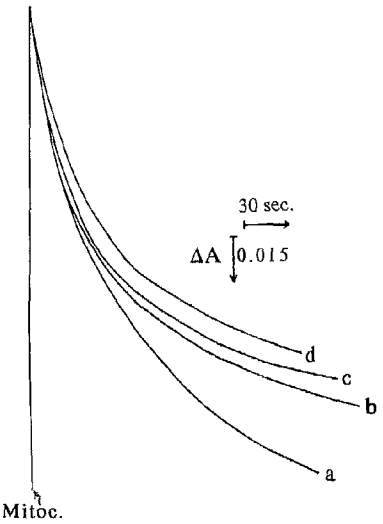


Fig. 11. Effect of DDT on swelling of mitochondria suspended in isoosmotic ammonium phosphate. Mitochondria (0.7 mg) were suspended in 3 ml of 135 mM ammonium phosphate, 5 mM Hepes, 0.1 mM EDTA, 2 μM rotenone (pH 7.2) at 25 °C. Tracings a to d were recorded in the presence of 0, 18, 36 and 70 nmol DDT/mg protein. Note that DDT decreases the rate of swelling induced by the entry of ammonium phosphate into mitochondria.

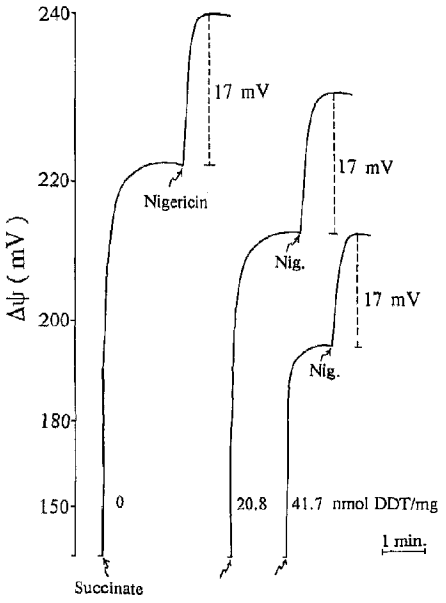


Fig. 12. Effect of nigericin on transmembrane potential ($\Delta\Psi$) of mitochondria energized with succinate, with or without DDT in the assay medium. Mitochondria (0.53 mg) in 1 ml of the standard respiratory medium were energized with 5 mM succinate; addition of nigericin (0.25 μg) promotes an increase of $\Delta\Psi$ not affected by the presence of DDT.

tive effects of DDT on the structural integrity of mitochondrial membranes.

Discussion

Previous reports of DDT on mitochondrial electron chain in insects [13] in rat heart [17] and in rat liver [18,19,29] are rather controversial. Byczkowski et al. [29] and Ohyama et al. [18] assigned uncoupling actions to DDT, whereas Nishihara [19] observed this uncoupling action at high concentrations of the pesticide during mitochondrial oxidation of ascorbate/TMPD. Contradictory results for the action of DDT on mitochondrial rat liver ATPase were also reported in addition to a well-defined inhibitory action of DDT on insect mitochondrial ATPase [30–32]. A DDT stimulatory action on rat liver mitochondrial ATPase was also reported by some authors [15], whereas others have observed inhibition [18].

The study of the mitochondrial transmembrane potential is essential for an integrated appraisal of the mitochondrial function, since it reflects basic useful energetic relationships. On the other hand, the $\Delta\Psi$ determinations are of major importance in studies of mitochondrial oxidative phosphorylation, since $\Delta\Psi$ represents the main component of the electrochemical gradient accounting for more than 90% of the total available energy [33].

Basically, the results achieved reflect specific actions of DDT within a limited range of concentrations above which unspecific effects related to membrane disruption may explain the uncoupling reported by several authors [15,16].

Due to the highly lipophilic character of DDT and its high partition coefficient, the observed effects of DDT depend on the insecticide/protein ratio rather than the absolute concentrations of the pesticide or the protein. Therefore, the reported conflicting effects might arise from the relative amounts used. Molar concentration of DDT is meaningless if the protein concentration is not stated. Data clearly show that the effects of the insecticide, up to a ratio of 50 nmol DDT/mg protein, are specific and not related with destruction of membrane organization. Therefore, comparison of results from different experiments is possible only if the ratios insecticide/protein are known and maintained.

The partition of DDT on mitochondrial membrane determined by Antunes-Madeira [34] is about 750000 (at 25 ° C), indicating a high incorporation of the pesticide preferentially occurring on the central core of the bilayer [35]. The high incorporation of DDT on mitochondrial membranes was related to its high fluidity resulting from the high content of unsaturated fatty acids and low content of cholesterol [34,36]. Therefore, the observed effect of DDT may result from a direct interaction with functional membranes proteins and/or from interactions with membrane phospholipids essential to the activity of the proteins. Actually, the Ca^{2+} pump activity of sarcoplasmic reticulum is affected by DDT probably through an interaction with the boundary lipids of the pump [37].

Independently of actions on the redox chain (ubiquinol-cytochrome *c* segment), DDT affects the phosphorylation system. This conclusion is further supported by the fact that succinate-cytochrome *c* reductase is inhibited by a mere 30% (40 nmol DDT/mg protein) whereas the phosphorylation rate is inhibited by 65% (Fig. 4). The significant effects of low DDT/protein ratios (3–6 nmol/mg) on ATP synthesis, but not on respiration (Fig. 4), further confirm the preferential interaction of DDT with the phosphorylation process. Additionally, depolarization induced by ADP is decreased by DDT, the lag phase preceding repolarization is extended and repolarization recovery is only partial (Figs. 7, 8). This effect results from direct insecticide action on ATP-synthetase since, in the presence of DDT, oligomycin added during the lag phase preceding repolarization fully restores the normal state 4 membrane potential (Fig. 8B). Apparently, DDT induces some sort of energy dissipation linked to ATP-synthetase by disrupting the coupling between F_0

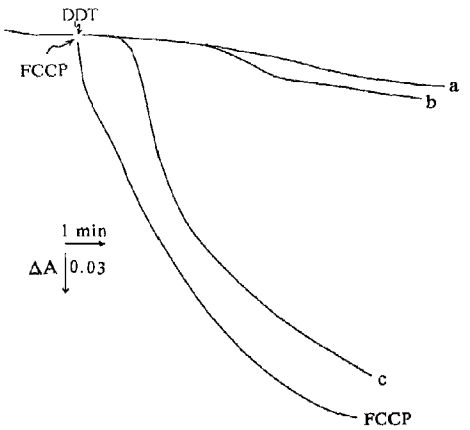


Fig. 13. Effect of DDT on swelling of mitochondria suspended in isoosmotic NH_4NO_3. Mitochondria (0.5 mg) were suspended in 3 ml of 135 mM NH_4NO_3, 5 mM Hepes, 0.1 mM EDTA (pH 7.2) at 25° C. DDT or FCCP were added after 6 min of incubation. Tracings a to c were recorded in the presence of 25, 38 and 88 nmol DDT/mg protein, respectively. Note that only high amounts of DDT promote permeabilization to H^+ due to destructive effects on the structural integrity of mitochondrial membranes.

and F_1. The direct action of DDT on ATP-synthetase was further confirmed from the effect on $\Delta\Psi$ associated with the ATPase activity of the complex, since $\Delta\Psi$ is decreased with a simultaneous increase of the ATPase activity (Fig. 9). Stimulation of ATPase activity is also observed in FCCP-uncoupled rotenone-inhibited preparations (not shown), indicating again that DDT interacts directly with the ATP-synthetase complex.

A classical uncoupling action of DDT can be ruled out on the basis of the following observations: (a) state 4 respiration is not significantly affected; (b) oligomycin inhibition of stimulated ADP respiration is not reverted by DDT; (c) mitochondria suspended in isoosmotic NH_4NO_3 do not swell, confirming that proton permeability is not affected (Fig. 13); (d) ΔpH is not altered by DDT (Fig. 12); (e) stimulation of ATPase activity in FCCP-uncoupled preparations. Patil et al. [32] have shown that DDT does not affect the F_1 component of ATP-synthetase (ATPase) and suggested that the pesticide may act on the enzyme by disrupting the bonding of OSCP (oligomycin sensitivity conferring protein) and F_1 or, alternatively, by perturbing the proteolipid components of F_0. This suggestion closely fits into our proposal for alteration of the functional link between F_0 and F_1 components of the ATP-synthetase complex.

The decrease of phosphorylation efficiency could also result from the partial inhibition of the carrier ADP-ATP or the transport of phosphate into mitochondria. According to the observations of Nishihara [19], the ADP-ATP carrier is not affected. To carefully ascertain this possibility, experiments with inside-out submitochondrial particles are currently underway and will be subject of an independent publication in the near future. A decreased membrane fluidity induced by DDT could partially explain the inhibition of phosphate carrier. Previous studies, however, indicate a fluidizing effect of DDT on mitochondrial membranes [35] and liposomes [11,35]. Therefore, the effect of DDT is presumably mediated by direct interaction with the carrier.

DDT may exert toxic effects by depressing the phosphorylation efficiency of mitochondria, causing severe impairment of energy requirements of the cell since mitochondria play a central role in cellular energy metabolism, supplying more than 90% of the total ATP required by the eucaryotic cell. Mitochondrial energy-dependent Ca^{2+} uptake is simultaneously affected (Fig. 10). Depression of $\Delta\Psi$ is expected to release intramitochondrial Ca^{2+} by the reversal of the electrogenic uniporter pathway [38], leading to a decrease in the mitochondrial Ca^{2+} concentration and an increase in the cytosolic Ca^{2+} level. The resulting perturbation on Ca^{2+}-sensitive enzymes [39] and Ca^{2+} homeostasis may result in severe cell injury [40].

According to Radomski et al. [41], exposed individuals could have accumulated about 20 nmol DDT/mg protein, assuming 16% protein in whole blood. However, this concentration and those used in our study cannot easily be correlated with LD_{50} for experimental mammals, since the distribution of DDT in tissues will depend on several parameters, namely the partition coefficients.

Acknowledgements

This work was supported by INIC and JNICT.

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