Adenosine Triphosphatase Activity in Brain, Intestinal Mucosa, Kidney, and Liver Cellular Fractions of the Red-Eared Turtle Following In Vitro Treatment with DDT, DDD, and DDE

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INTRODUCTION

Increasing awareness of the environmental effects of organochlorine insecticides has stimulated research on the effects of these compounds on active transport across cellular membranes. The effects of DDT on the ATPase systems of liver, intestinal mucosa, cloacal bladder, and kidney tissue of 5 turtle species were studied by PHILLIPS and WELLS (1974). Their data indicated that the total (Na^+, K^+, Mg^{+2})-, Mg^{+2}-, (Na^+, K^+)-dependent ATPases in the tissues of all 5 turtle species were significantly inhibited by 53 uM DDT. There was some inhibition and stimulation following treatment with 5.3 uM DDT.

The purpose of this study was to determine the effect of various concentrations of DDT, DDD, and DDE on the ATPase systems in cellular fractions of tissues from the red-eared turtle, Chrysemys scripta elegans.

METHODS

Adenosine triphosphatase (ATPase) activity in cellular fractions of turtle tissues was determined by measuring the amount of inorganic phosphate produced by the conversion of adenosine triphosphate to adenosine diphosphate.

One turtle species was assayed for ATPase activity: the red-eared turtle, Chrysemys scripta elegans. All turtles were trapped in middle and west Tennessee and placed in plastic swimming pools 1.5 m in diameter. Water levels were kept at 6 cm, and room temperature ranged from 23° to 27° C.

Each turtle was anesthetized with ethyl ether until it was limp (30-60 min). The plastron was then surgically removed with a Weber bone saw and portions of the kidney, liver, and intestine removed and placed on cold aluminum foil. The dorsal skull was excised and the brain removed and placed on cold aluminum foil. Each tissue sample was weighed immediately and added to an ice cold solution containing 0.25 M sucrose, 0.005 M disodium ethylenediamine

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tetraacetic acid, and 0.03 M histidine buffer (pH 7.4) to yield a 5 percent W/V concentration (JANICKI and KINTER, 1971). The tissues were homogenized in Ten Broeck glass homogenizers in ice. Differential centrifugation, according to the method of WELLS and YARBROUGH (1972) was carried out in a Beckman Ultracentrifuge Model L3-50, with Type SW 50.1 rotor. Cellular fractions included nuclei, cell membranes, mitochondria, heavy microsomes, and light microsome-soluble portions for all tissues, as well as a myelin cellular fraction from brain tissue.

Pellets of these cellular fractions were resuspended in 3 ml of the incubation medium which contained 20 mM histidine buffer (pH 7.4), 100 mM NaCl, and 20 mM KCl (JANICKI and KINTER, 1971). A 0.10 ml aliquot of this suspension was added to 4.25 ml of the incubation medium. Each sample also contained either 0.25 ml of 5 percent N, N-dimethylformamide (DMF), or DDT, DDD, or DDE concentrations of 53 uM, 106 uM, and 212 uM in the reaction mixture. Samples were assayed in triplicate. Samples were incubated for 30 minutes, which allowed the DDT, DDD, or DDE to pervade the homogenate. The reaction was initiated by addition of 50 ul of 100 mM Na_2ATP and 100 mM $MgCl_2$ and continued for 30 minutes at 24° C. ATPase activity was terminated by the addition of 1 ml of ice cold 30 percent trichloroacetic acid. To allow complete percipitation of the homogenate proteins, the samples were placed in a freezer (0° C) for 30 minutes.

DDT, DDD, and DDE were extracted from each sample using equal amounts of ice cold hexane to preclude density interference in the colorimetric determination of inorganic phosphate by insoluble suspended crystals. Samples were stored overnight to facilitate separation of the homogenate from the hexane. The unfrozen top layer of DDT (DDD, DDE)/hexane was decanted and the homogenate thawed to room temperature.

Inorganic phosphate produced as a result of the cleavage of ATP to ADP was measured by the method of FISKE and SUBBAROW (1925) as modified by BARTLETT (1958). Color development proceeded at room temperature for 10 minutes. The optical density of each sample was measured with a Brinkmann PC/1000 colorimeter at 670 nm.

The ATPase activity was determined for total (Na^+, K^+, Mg^{+2})-dependent ATPase. Significant differences were determined at the 0.05 (significant) and 0.01 (highly significant) level of confidence using the t test.

RESULTS

There was highly significant inhibition of the (Na^+, K^+, Mg^{+2})-dependent ATPase systems in all cellular fractions of the brain (except mitochondria) after in vitro treatment with 212 uM DDT (Fig. 1).

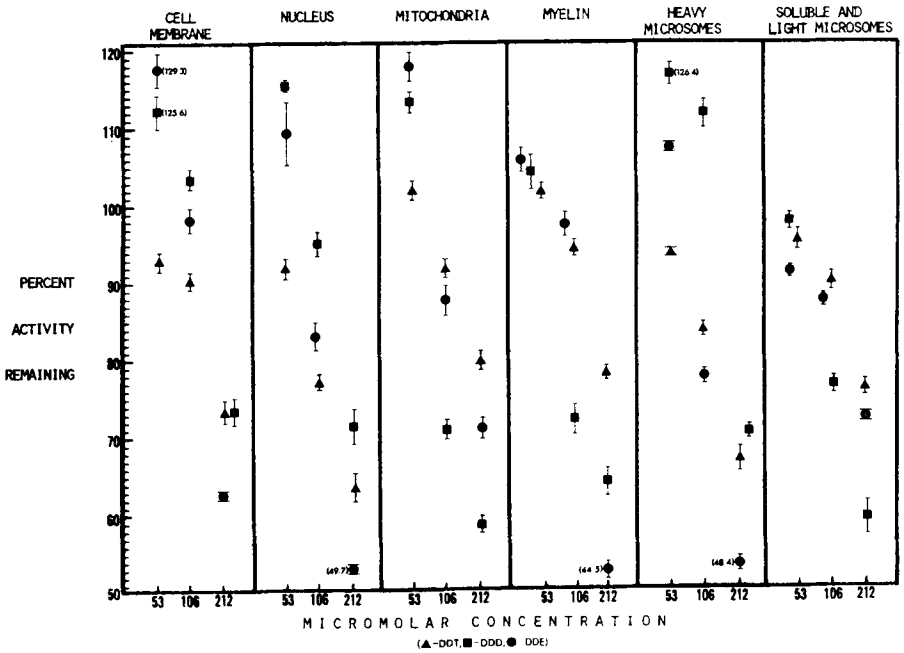

Fig. 1. Activity of (Na^{+}, K^{+}, Mg^{+2})-dependent ATPase of the cellular fractions of the brain following in vitro treatment (30 min) with DDT, DDD, and DDE. Values are expressed as percent of original activity remaining $\pm$ SEM.

The addition of 106 uM DDT resulted in highly significant inhibition of brain nuclei and heavy microsomes, while a concentration of 53 uM DDT had little effect on brain cellular fractions. Significant inhibition of brain cell membranes and soluble and light microsomes, as well as highly significant inhibition of all other fractions, occurred at 212 uM DDD. A concentration of 106 uM DDD had little effect on cell membranes and nuclei, but resulted in significant inhibition of mitochondria, myelin, and soluble and light microsomes. There was highly significant stimulation of heavy microsomes at 106 uM and 53 uM DDD. Addition of 53 uM DDD also resulted in significant stimulation of nuclei, cell membranes, and mitochondria. In vitro treatment with 212 uM DDE resulted in highly significant inhibition of all cellular fractions of the brain. A concentration of 106 uM DDE had similar effects, except in myelin and cell membranes, where little effect was noted. Significant stimulation in all fractions except myelin followed addition of 53 uM DDE. The greatest amount of inhibition of brain cellular fractions occurred in myelin after treatment with 212 uM DDE, where 44.5 percent activity remained. The cell membranes showed the greatest stimulation of all brain cellular fractions (129.3 percent activity remaining) after in vitro addition of 53 uM DDE.

There was highly significant inhibition of all cellular fractions of the intestinal mucosa (except mitochondria) after

treatment with 212 uM DDT (Fig. 2). Significant inhibition resulted at

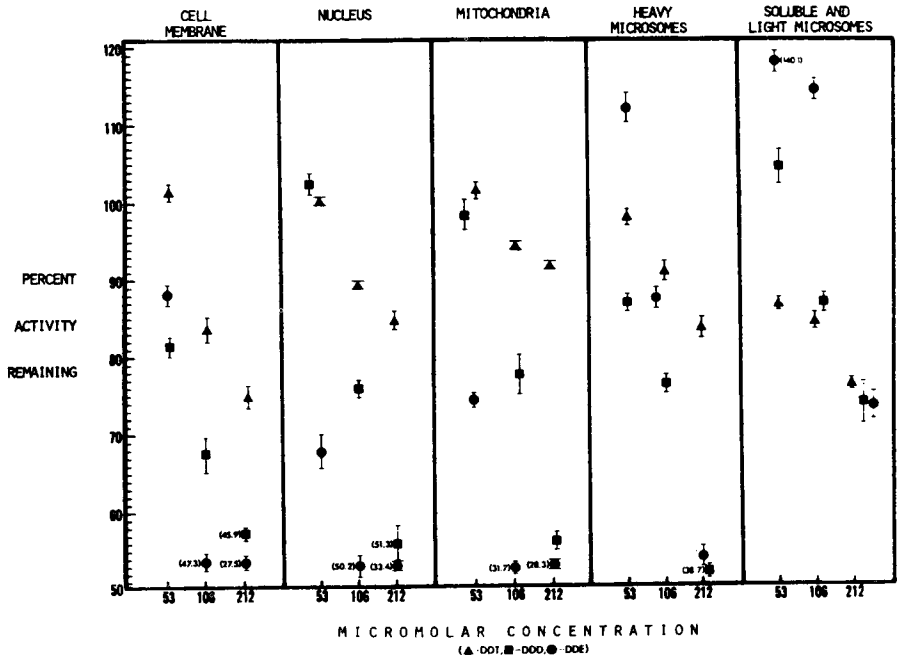

Fig. 2. Activity of (Na^+, K^+, Mg^{+2})-dependent ATPase of the cellular fractions of the intestinal mucosa following in vitro treatment (30 min) with DDT, DDD, and DDE. Values are expressed as percent of original activity remaining ± SEM.

106 uM DDT in cell membranes, nuclei, heavy microsomes, and soluble and light microsomes. A concentration of 53 uM DDT had little effect on the intestinal mucosa, except in the soluble and light microsomes, which were inhibited to a highly significant degree. Significant inhibition of all cellular fractions of the intestinal mucosa occurred after in vitro additions of 106 uM and 212 uM DDD and DDE. Treatment with 53 uM DDD caused highly significant inhibition of heavy microsomes, but had little effect on other cellular fractions. While 53 uM DDE had little effect on cell membranes, there was significant inhibition of nuclei, mitochondria, and heavy microsomes, and highly significant stimulation of soluble and light microsomes. Of the intestinal cellular fractions, the greatest inhibition occurred in the cell membranes at 212 uM DDE, which retained 27.5 percent of original enzyme activity. The greatest stimulation, 140.1 percent activity remaining, was caused by a concentration of 53 uM DDE and occurred in the soluble and light microsomes.

The in vitro addition of 106 uM and 212 uM DDT caused significant inhibition in all cellular fractions of the kidney (Fig. 3). A concentration of 53 uM DDT resulted in some inhibition

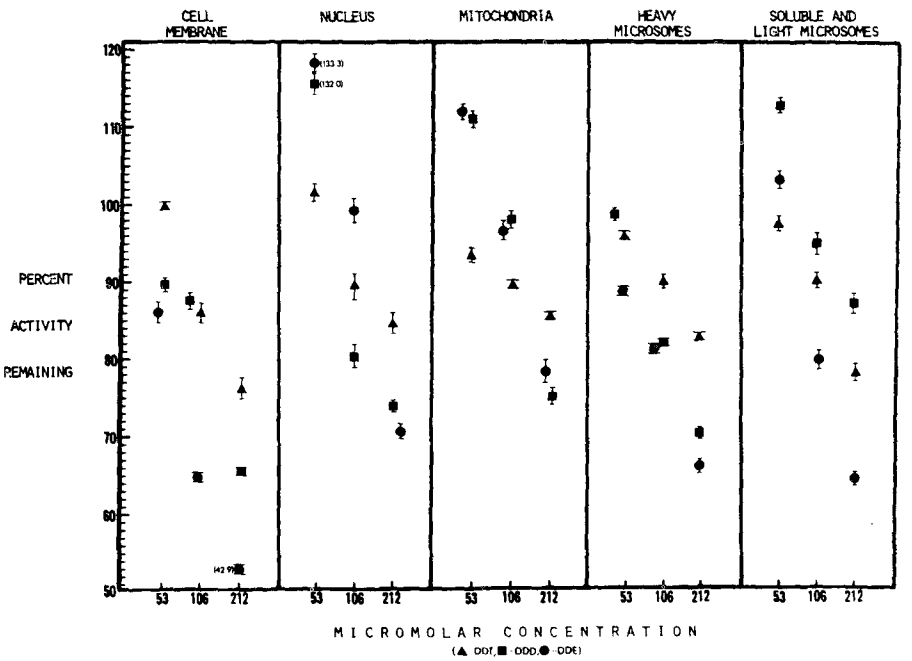

Fig. 3. Activity of (Na^+, K^+, Mg^{+2})-dependent ATPase of the cellular fractions of the kidney following in vitro treatment (30 min) with DDT, DDD, and DDE. Values are expressed as percent of original activity remaining $\pm$ SEM.

of mitochondria and heavy microsomes, but had no apparent effect on other kidney cellular fractions. Treatment with 212 uM DDD resulted in highly significant inhibition of all fractions except mitochondria, where there was minimal effect. Significant inhibition of nuclei and heavy microsomes occurred at 106 uM DDD, and significant stimulation of nuclei and soluble and light microsomes occurred at 53 uM DDD. A concentration of 212 uM DDE caused highly significant inhibition of all kidney cellular fractions except the mitochondria, which were only moderately inhibited. The addition of 106 uM DDE had no apparent effect on nuclei and mitochondria, but caused highly significant inhibition of all other cellular fractions. At 53 uM DDE, there was a high degree of stimulation of the nuclei and highly significant inhibition of cell membranes and heavy microsomes. Addition of 53 uM DDD or DDE to the nuclei caused the greatest stimulation of all cellular fractions with about 132 percent activity remaining in each case. The greatest inhibition of all fractions was seen in the cell membranes after in vitro addition of 212 uM DDE, where

43 percent of original enzyme activity remained.

Following in vitro addition of 212 uM DDT, all cellular fractions in the liver were inhibited to a highly significant degree (Fig. 4). A similar effect was seen at 106 uM DDT, except

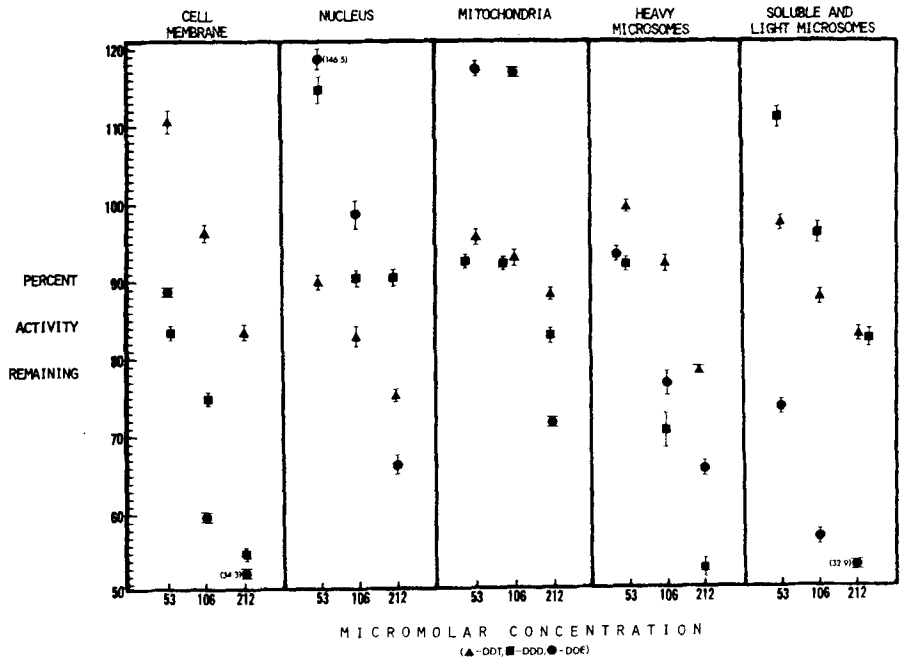

Fig. 4. Activity of (Na^+, K^+, Mg^{+2})-dependent ATPase of the cellular fractions of the liver following in vitro treatment (30 min) with DDT, DDD, and DDE. Values are expressed as percent of original activity remaining ± SEM.

that the mitochondria were inhibited to a lesser extent, and the cell membranes were little affected. A concentration of 53 uM DDT had no apparent effect, except for a slight, though significant, inhibition in nuclei and mitochondria. After addition of all concentrations of DDD to liver cell membranes, there was highly significant inhibition. Concentrations of 106 uM and 212 uM DDD caused a highly significant degree of inhibition in heavy microsomes. The soluble and light microsomes of the liver were significantly stimulated at 53 uM DDD and showed highly significant inhibition at 212 uM DDD. All cellular fractions of the liver exhibited a high degree of inhibition following in vitro addition of 212 uM DDE. A highly significant inhibition occurred at 106 uM DDE in all fractions except nuclei, which were apparently not affected. The

addition of 53 uM DDE caused a highly significant stimulation of nuclei and mitochondria and a highly significant inhibition of cell membranes and soluble and light microsomes. A concentration of 53 uM DDE had little effect on heavy microsomes of the liver. The greatest stimulation of liver cellular fractions occurred in the nuclei after treatment with 53 uM DDE (146 percent). The greatest degree of inhibition was seen in the cell membranes and soluble and light microsomes following addition of 212 uM DDE. In each case, about 33 percent of original enzyme activity remained.

It is important to note an inverse relationship existed between percent enzyme activity remaining and concentrations of DDT, DDD, and DDE. The DDT analogue, DDE, caused both the greatest stimulation and greatest inhibition in each tissue studied. The effects of DDT on the (Na^+, K^+, Mg^{+2})-dependent ATPase systems were not as great as those of DDD or DDE.

DISCUSSION

Active transport systems serve to concentrate nutrients within the cell, to maintain the proper level of inorganic electrolytes, and to maintain the correct osmotic pressure and volume of intracellular fluid. These activities require the passage of solutes against a concentration gradient and are primarily dependent on ATP for energy. When ATP is hydrolyzed by the action of ATPase, ADP and inorganic phosphate are produced, and energy, from the high energy phosphate bond, is released. This energy is utilized to drive the active transport systems.

The inhibition of (Na^+, K^+, Mg^{+2})-dependent ATPase system by DDT was expected. However, the degree of inhibition by DDE was not expected. It is interesting to note that DDT does not share the sites of greatest inhibition with either DDT or DDE, suggesting a different mechanism of action. It seems likely that the normal functions of these tissues and the concomitant recovery of the cell would be greatly affected by such concentrations of DDT, DDD, and, especially DDE.

When ingested in trace amounts, DDT is concentrated and stored in fatty tissues at a rate of ten to twenty times that of ingestion. These storage deposits are slowly converted to DDE and ultimately excreted as DDA (METCALF, 1971). At sublethal pesticide levels, laboratory studies have shown that there is impairment of mechanisms which are critical for survival. When rainbow trout containing endrin residues were subjected to forced swimming, the blood cortisol decreased rapidly, and liver glycogenolysis was inhibited. Dieldrin yielded similar observations (MENZIE, 1972). An animal with irregular food intake, or in a state of starvation, may depend on its fat reserves for energy. In such animals with accumulations of DDT and DDE (and presumably DDD) in their fat deposits, there would be a constant release of toxic material as the fats are metabolized, and organ functions may be impaired. If a stress situation is then encountered, the animal with such function already impaired may not be able to survive the stress. Thus, the effects of DDT and its degradation products, DDD and DDE, might, under these conditions, be lethal.

DDT, DDD, and DDE are stored in fatty tissue and, presumably the fat portion of cellular membrane structure. Since ATPase is a structural part of these membranes, it is possible that DDT, DDD, and DDE act to alter the configuration of the ATPase molecule, thereby affecting its active site and its activity. In such a case, energy production would be curtailed, and cellular functions inhibited.

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