ATPase Activity in Tissues of the Map Turtle, *Graptemys geographica* following *in vitro* Treatment with Aldrin and Dieldrin

by

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Interest in the environmental impact of organochlorine insecticides has led to numerous studies concerning the effect of these compounds on active transport across cellular membranes. One of the initial efforts to specifically explain the action of DDT in active transport was by MATSUMURA et al. (1969). By using differential centrifugation techniques, they isolated various nerve components of rat brain and localized the source of ATPase sensitive to DDT. Their results indicated that (Na^+, K^+, Mg^{++})-dependent ATPase in the rat brain is specifically sensitive to DDT. They suggested that DDT is causally related to disruption of ion transport mechanisms in the nervous system in vivo.

Interest in mechanisms of reciprocal transfer of Na^+ and K^+ across the plasma membrane of individual cells caused JAMPOL AND EPSTEIN (1970) to investigate the specific activity of (Na^+, K^+)-dependent ATPase in gill, intestine, and kidney tissues of the American eel, Anguilla rostrata. Their data indicate that (Na^+, K^+)-dependent ATPase plays an important role in active transport of sodium across epithelial membranes. Using similar methods, JANICKI AND KINTER (1971) conclusively showed that DDT inhibits (Na^+, K^+, Mg^{++})-dependent ATPases engaged in active sodium transport functioning to maintain tissue osmolarity. In several species of intact fish, 10 ppm DDT or less was lethal. In their study, 1 to 10 ppm DDT in the in vitro assay caused a 40% inhibition of the enzyme. They also found that cyclohexanone (a commercial DDT solvent) completely inhibited (Na^+, K^+, Mg^{++})-dependent ATPases.

In long-term exposure of organochlorine compounds in vivo, (Na^+, K^+, Mg^{++})-dependent ATPases were inhibited in several fish species (KOCH et al. 1972). In some cases, notably at the lower concentrations of DDT, erratic stimulation occurred. Stimulation was most pronounced in kidney and liver tissues.

The purpose of this study was to determine the effect of various concentrations of aldrin and dieldrin upon the ATPase system in the map turtle, Graptemys geographica.

MATERIALS AND METHODS

Activity of adenosine triphosphatase (ATPase) was determined in turtle tissues by measuring the amount of inorganic phosphate produced when adenosine triphosphate was converted to adenosine diphosphate.

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Utilizing procedures adapted from current literature, appreciable errors were observed when using relatively large volumes of ATP with standard phosphate. Excess amounts of ATP inhibit the color complex formed between molybdate and inorganic phosphate using the Fiske and SubbaRow method. MARSH (1958) concluded that the inhibition of this reaction was due to the molybdate-catalyzed hydrolysis of excess ATP during color development. Marsh's warning concerning the use of excess ATP has apparently been ignored by several contemporary researchers.

The map turtle, Graptemys geographica was trapped in the Reelfoot Lake area of West Tennessee. Each turtle was placed in plastic swimming pools 1.5 m in diameter. Water levels were maintained at 5 cm in the storage pools. Room temperature ranged from 23 to 27°C. Animals were exposed to a diurnal cycle of natural lighting in conjunction with a timed artificial lighting system. All map turtles assayed were females.

Each turtle was anesthetized with ethyl ether until it was limp (30 to 60 min). The plastron was surgically removed with a Weber bone saw and a portion of the liver, kidney, cloacal bladder, and intestinal mucosa was removed and weighed. Each tissue sample was transferred to a cold solution containing 0.25 M sucrose, 0.005 M disodium ethylenediamine tetraacetic acid, and 0.003 M histidine buffer (pH 7.4 to yield a 5% w/v concentration). Tissues were homogenized in a Ten Broeck glass homogenizer in ice. Activity of (Na^+, K^+, Mg^{++})-dependent ATPase was determined at 21 to 24°C. Glassware was washed immediately after each determination with distilled deionized water and the Fiske and SubbaRow reagents. After each fifth determination, the glassware was washed with hot 10 N HCL. A 0.2 ml aliquot of the tissue homogenate was added to 4.25 ml of the incubation media containing 20 mM histidine buffer (adjusted to pH 7.4), 100 mM NaCl, and 20 mM KCL. Each sample also contained either 0.25 ml of 5% N, N-dimethylformamide (DMF) or aldrin and dieldrin concentrations of 1 mM, 0.1 mM, and 0.01 mM in DMF, giving a pesticide concentration of 53 uM, 5.3 uM, and 0.53 uM in the reaction mixture. Samples were assayed in triplicate. Samples were incubated for 30 min. to allow the aldrin or dieldrin 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 with agitation for 30 min. at 24°C. ATPase activity was terminated by the addition of 1.0 ml of ice cold 30% trichloroacetic acid. Samples were then transferred for 30 min. to a refrigerator (5°C) to allow complete precipitation of the homogenate proteins. The precipitate was sedimented in an International clinical centrifuge at 3,000 rpm for 3 min.

Aldrin and dieldrin was 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 at 0°C to facilitate separation of the homogenate from the hexane. The unfrozen top layer (hexane/pesticide) 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 min. The optical density of each sample was measured with a Beckman Spectronic 70 spectrophotometer at 660 nm. The comparison of ATPase activity between turtles was based on homogenate protein (method of LOWRY et al. 1951). Statistical analysis was by the t test.

RESULTS AND DISCUSSION

Inhibition of total (Na^+, K^+, Mg^{++})-dependent ATPase occurred in all tissues at 53 uM, 5.3 uM, and 0.53 uM aldrin (Fig. 1). The greatest inhibition was in the intestinal mucosa treated with 53 uM aldrin. The inhibition was not as pronounced at lesser concentrations of aldrin.

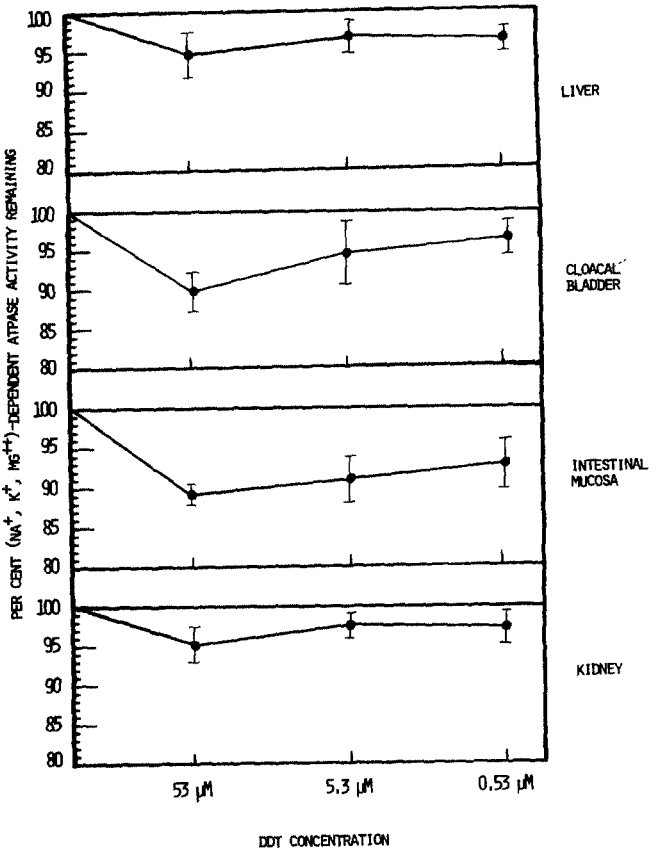

Figure 1. Percent (Na^+, K^+, Mg^{++})-dependent ATPase activity remaining in tissues from the map turtle following treatment with aldrin. Values expressed as the mean ± SE.

The effect of dieldrin on the ATPase system was greater than aldrin except in the intestinal mucosa (Fig. 2). The greatest inhibition by dieldrin was in the cloacal bladder at 53 uM.

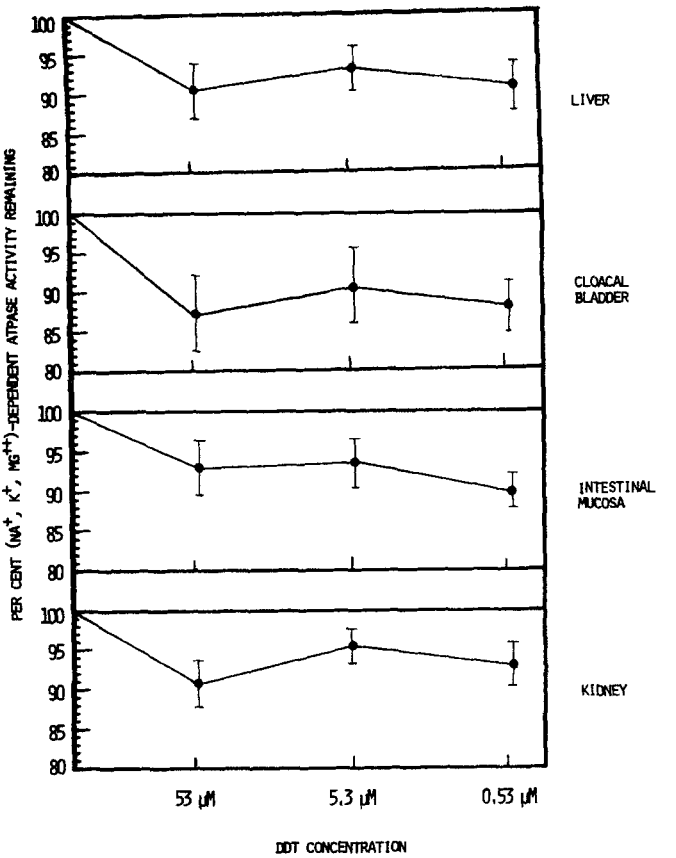

Figure 2. Percent (Na^+, K^+, Mg^{++})-dependent ATPase activity remaining in tissues from the map turtle following in vitro treatment with dieldrin. Values expressed as the mean $\pm$SE.

The amount of inhibition present in the cloacal bladder and intestinal mucosa of all species indicates that aldrin and dieldrin may have an effect upon absorption of metabolites due to a lack of energy required for active transport across the cellular membrane. In this study there was inhibition of intestinal mucosa and cloacal bladder ATPase after in vitro treatment with aldrin and dieldrin.

It is possible that treatment with equal concentrations of aldrin and dieldrin in vivo would cause an inability to effectively utilize metabolites.

The observed action of aldrin and dieldrin in all tissues assayed might be related to the ability of the compound to alter the cellular membrane configuration by binding with the fat portion of the membrane. Since ATPase is a structural part of the membrane, the active site of the enzyme would be altered. Thus, the energy needed to "pump out" the pesticide would be reduced. Movement of other substances by active transport would likewise be reduced. If the uptake of aldrin and dieldrin by turtles in a natural environment reaches tissue concentrations equal to those found in this study, the resulting ATPase inhibition may be sufficient to reduce organ functions.

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