EFFECT OF BIS-TRIBUTYLTIN OXIDE ON THE RECTAL GLAND OF SQUALUS ACANTHIAS.

P. Silva, P. Silva, Jr., J.P. Silva, M. Silva, R. Cohen, S. Lear, K. Spokes, R. Solomon, and F.H. Epstein. Department of Medicine Beth Israel Hospital Harvard Medical School and Medical College of New York.

The organotin most widely used as the active ingredient of antifoulant marine paints is bis(tributyltin) oxide (TBTO). We report here preliminary studies of the effect of TBTO on the oxygen consumption of isolated rectal gland cells, and the activities of Na-K-ATPase and proton (oligomycin sensitive) ATPase.

Isolated rectal gland cells were prepared as previously described. The glands were then perfused with 10 ml of shark Ringer's containing 0.2% collagenase, 0.25% hyaluronidase and 10% fetal calf serum. After perfusion was stopped the glands were sectioned longitudinally and minced into 0.5 to 1 mm cubes. The minced tissue was placed in shark Ringer's containing collagenase and 10% fetal calf serum, at room temperature, while constantly stirring, for 45 minutes. The tissue digest was centrifuged at 50 x g for 1 min in a refrigerated centrifuge to remove the undigested tubules; and the supernatant was then centrifuged at 500 x g for three minutes to harvest the cells. The cells were washed twice with shark Ringer's and kept on ice until used.

Oxygen consumption was measured in a constant temperature (25°C) chamber using a Clark type polarographic oxygen electrode (YSI) connected to a recorder. Glucose 5 x 10^{-3}M, pyruvate 10^{-2}M and acetate 2.5 x 10^{-3}M were added together as exogenous substrates. All additions to the measuring chamber were made in volumes of less than 1% of the volume of the chamber. Results are expressed as micromoles of O_2 consumed per hour per gram wet weight of cells.

Table I shows the effect of TBTO on oxygen consumption of isolated rectal gland cells. TBTO inhibited basal oxygen consumption at all concentrations studied, and prevented the stimulation of respiration induced by 0.25 mM theophylline in combination with 1 mM dibutyryl cyclic AMP at concentration of 10^{-9}M and higher. At concentration of 10^{-7}M it abolished cellular respiration. In the experiment in which TBTO was added at a concentration of 10^{-7}M, 2,4-dinitro phenol 5 x 10^{-5}M did not increase cellular respiration indicating that TBTO had irreversibly damaged the mitochondria of the cells.

Na-K-ATPase and proton ATPase activity were determined enzymatically by the method of Bartlett in the presence and absence of ouabain 10^{-4}M with and without oligomycin 10μg/ml. Results are expressed as μmoles of ATP hydrolized per mg of protein per hour.

The effect of TBTO on Na-K-ATPase was tested at concentrations of TBTO ranging from 10^{-10}M to 10^{-7}M. The enzyme was inhibited maximally at a concentration of TBTO of 10^{-10}M. Lower concentration of the organotin were not tested. Proton ATPase (oligomycin sensitive) was inhibited in a dose dependent way starting at a concentration of 10^{-10}M with maximal inhibition at 10^{-8}M. Residual ATPase, 10% of total ATPase, was not affected by the organotin.

These results indicate that TBTO alters cellular metabolism and consequently cellular function by interfering with enzymatic activity and mitochondrial respiration. Its is likely that the inhibition of chloride transport is the result of the non-specific effect of TBTO on cellular respiration and enzymatic activity. TBTO causes liver and kidney alterations and mitochondrial damage in a number of fish species at concentrations similar to those used in these studies (Biochem J 61:406, 1955; J Fish Biol 10:575, 1977; Sci Total Environ 19:155, 1981). The amount of TBTO required to cause tissue damage and functional alterations is within the range reported to be present in areas where antifoulant paints are used (Marine Pollution Bull 14:303, 1983). TBTO should therefore be considered as a dangerous pollutant capable of causing serious damage to the marine environment and potentially causing even greater damage if it enters the food chain.

Table I

Effect of tributyltin on oxygen consumption in isolated rectal gland cells.

	Basal	Tributyltin	db cAMP 1 mM theophylline 0.25 mM
Tributyltin			
10^{-7}M	20.3(1)	2.24(1)	
10^{-8}M	42.4±6.5(3)	7.0±1.7(3)	NC
10^{-9}M	37.9±8.2(7)	8.7±2.5(7)	NC
10^{-10}M	27.7±8.9(5)	9.9±2.9(5)	
10^{-11}M	21.5±3.8(4)	12.4±2.7(4)	21.6±4.7(4)
10^{-12}M	31.8±2.7(7)	14.6±1.3(6)	26.0±2.5(6)
10^{-13}M	38.6±6.2(5)	21.3±3.9(5)	34.3±6.2(5)
10^{-14}M	23.7±2.6(5)	15.4±2.1(5)	27.9±5.8(5)
10^{-15}M	19.8±4.8(5)	13.2±3.8(4)	22.5±3.9(4)
10^{-16}M	18.8±1.3(3)	11.6±1.6(3)	13.5±3.5(3)

Values are mean±SEM expressed in μM O_2 utilized per min per gram wet weight.
NC = no change in oxygen consumption after TBTO.