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## INSECTICIDE IMPACT ON CONTRACTILE PATTERN OF AMPHIBIAN SKELETAL MUSCLE

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### ABSTRACT

Effect of different concentrations of organochlorine pesticide (DDT) on twitch properties of frog gastrocnemius muscle showed significant decrease in the shortening length, twitch duration, and half contraction time (HCT), and half relaxation time (HRT), during direct (DS) and indirect (IDS) stimulations, indicating inhibitory modulation of DDT on contractile kinetics of the normal muscle.

### INTRODUCTION

**T**HOUGH organochlorine pesticide DDT has been extensively used, the detailed mechanism of its action and toxicity remains obscure<sup>1</sup>. DDT is primarily a neurotoxicant which inhibits the acetylcholinesterase activity, leading to atoxia, convulsion and eventual paralysis<sup>2,3</sup>. It has also been reported that DDT increases the nerve acetylcholine, not by inhibiting the enzyme but by enhancing the liberation of neurotransmitter from bound reserves, which play a dynamic role in the contraction characteristics of the excitable tissues<sup>4</sup>. Since DDT is known to inhibit ATPase reacting system<sup>5</sup> and oxidative metabolism<sup>6,7</sup> involved in the energy production of the contractile machinery, an attempt is made in the present investigation, to study the specific modulatory effect of this organochlorine pesticide, on the twitch properties and related contraction kinetics in the skeletal muscle of the frog.

### MATERIALS AND METHODS

Healthy medium sized frogs, *Rana hexadactyla* were double pithed and the gastrocnemius muscles from both the legs were excised with least injury.

The muscles were washed 3 to 4 times in amphibian Ringer<sup>8</sup> and allowed to stand in fresh Ringer medium for 10 minutes to recover from the shock effects. Single muscle twitches were recorded before and after presoaking the muscle for 10 minutes in 20, 40, 60 and 80 ppm DDT mixed in Ringer solution as per the method suggested by Matzaeman Uchida<sup>9</sup>. Single stimulus of 6 volts D.C. was found to elicit optimal response for both DS and IDS; this voltage was considered to be the threshold voltage. The twitch properties and the associated contraction kinetics were analysed by using a calibrated kymographic unit<sup>10</sup>. Contractile patterns of the muscle were recorded on smoked paper and the recordings were made permanent in 2:1 turpentine-varnish mixture. The amplitude of contraction or shortening length, twitch duration, HCT and HRT were calculated. Average values of six analyses were considered for the present study.

### RESULTS AND DISCUSSION

The twitch properties of gastrocnemius muscle subjected to DS and IDS were analysed from the contraction pattern recordings (Table I).

When compared to DS, the HCT and HRT decreased during IDS, indicating that IDS elicits quicker

TABLE I  
Amplitude, Twitch duration, HCT, HRT of the muscles presoaked in different concentrations of DDT Ringer solutions

Sl. No.	Concentration of DDT in ppm	Direct stimulation					Indirect stimulation				
		HCT (m. sec)	HRT (m. sec)	HRT/HCT	Amplitude (cm)	Twitch duration (m. sec)	HCT (m. sec)	HRT (m. sec)	HRT/HCT	Amplitude (cm)	Twitch duration (m. sec)
1.	0.0 (Control)	47.4 ±1.9	59.7 ±2.2	1.2	3.6 ±0.1	214.2	42.9 ±2.1	57.1 ±2.6	1.3	3.4 ±0.1	200.0
2.	20	43.2 ±1.3 P<0.001	60.8 ±2.8 NS	1.4	3.2 ±0.1 P<0.001	208.1	26.8 ±3.2 P<0.001	35.0 ±2.1 P<0.001	1.3	2.8 ±0.2 P<0.001	123.0
3.	40	32.9 ±1.4 P<0.001	49.4 ±1.9 P<0.001	1.5	2.2 ±1.0 P<0.001	164.8	20.6 ±2.9 P<0.001	30.9 ±2.5 P<0.001	1.5	1.8 ±0.1 P<0.001	103.0
4.	60	28.8 ±1.6 P<0.001	45.3 ±2.0 P<0.001	1.5	1.4 ±0.2 P<0.001	148.3	14.4 ±1.5 P<0.001	28.8 ±2.8 P<0.001	2.0	1.1 ±0.3 P<0.001	86.5
5.	80	18.5 ±1.1 P<0.001	30.9 ±1.7 P<0.001	1.6	0.7 ±0.1 P<0.001	98.9	12.3 ±1.4 P<0.001	26.8 ±1.1 P<0.001	2.2	0.3 ±0.0 P<0.001	78.3

Values are mean, ± S.D. of six observations ; HCT : Half contraction time ; HRT : Half relaxation time.

TABLE II

Per cent difference of various contraction kinetic parameters for IDS over DS of the muscles presoaked in different concentrations of DDT-Ringer solutions.

Sl. No.	Concentration of DDT in ppm	HCT	HRT	HRT/HCT	Amplitude	Twitch duration
1.	0.0 (control)	-9.3	-4.4	+5.1	-5.5	-6.6
2.	20	-38.1	-42.4	-6.9	-12.5	-40.9
3.	40	-37.5	-37.5	0.0	-18.2	-37.5
4.	60	-50.0	-36.3	+27.3	-21.4	-41.6
5.	80	-33.5	-14.3	+30.0	-50.0	-20.8

contractile response from the muscle in comparison with that of DS. The HRT/HCT showed an increase in IDS, suggestive of greater decrement of HCT than HRT. There was no significant change in the amplitude of contraction both in DS and IDS. Hence, it is presumed that the IDS confers relatively more efficient contraction potential on the muscle than the DS.

When the muscles were presoaked in DDT solutions of different concentrations ranging from 20 to 80 ppm, the contraction kinetics were altered. The HCT and HRT showed continuous decrement with the increase in the concentration of DDT during both types of stimuli. But, the decrement is more in IDS than in DS (Table II), indicating shortening of the period of contraction and relaxation cycle. However, it does not indicate quicker and more efficient pattern of contraction as the amplitude of the contraction showed a decrement. The HRT/HCT ratios indicated an increase with the DDT concentration in the medium, suggesting that the contraction phase of the muscle on IDS was more affected than the relaxation phase by this organochlorine pesticide as compared to DS (Table II). The per cent difference in the amplitude also showed steady negative values which increased proportionately to the DDT concentration, revealing greater susceptibility of IDS to DDT than DS.

Since the contraction kinetics of IDS is more affected than that of DS, the DDT inhibitory modulation has a greater effect on the contraction pattern elicited through neuro-muscular junction than through individual cellular response systems. It may also be said that, the DDT, perhaps interferes with the acetylcholine esterase (AChE) system at the neuro-muscular junction by reducing AChE activity and thereby elevating the nerve acetylcholine or by enhancing the liberation of acetylcholine from the bound reserves<sup>6</sup>. In

general, it can be stated that this change in the contraction potential of DDT presoaked muscle may be possible due to the lower availability of carbohydrate reserves<sup>6</sup>, or due to the inactivation of enzymes, involved in energy yielding systems such as SDH<sup>11,12</sup>, Cytochrome oxidase and ATPase<sup>5-7</sup>.

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