capsule around the fluke is made up of fibrous connective tissue and the fibres stain blue with Mallorys' triple stain. In infected fishes there is oedema of submucosa and the blood supply of the area has been increased. There is vasodilation and the blood capillaries are congested in these fishes. Lymphoidal tissue of 0·135 mm 0·306 mm diameter is seen in the stomach wall of *C. gachua* (Ham.) near the capsule containing *G. goppo*. However, the muscular layers and serosa are not affected by infection with *G. goppo*.

Effects of trematode infection in fishes have not yet been properly studied (Rogers[2]), though such work has been done in some birds and domestic mammals (Campbell and Jackson[1]).

This is the first report of effects of trematode infection in fishes.

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1. Campbell, N. J. and Jackson, C. A. W., *Aust. Vet. Jour.*, 1977, p. 29.
2. Rogers, W. A., *Proc. 3rd International Wild Life Diseases Conference*, 1976, p 153.

MALATHION IMPACT ON CONTRACTION KINETICS OF AMPHIBIAN SKELETAL MUSCLE

MALATHION, (0,0-dimethyl S-(1,2-dicarbethoxyethyl) was known to disrupt neural transmission in vertebrates by inhibiting acetylcholinesterase enzyme that modulates the amounts of neurotransmitter acetylcholine[1–7]. The studies of Murphy[4] and Murphy *et al.*[8] showed that malathion has very little or indirect effect on acetylcholinesterase. The inhibitory modulation of malathion toxicity on the muscle twitch characteristics can be understood well by taking a vertebrate skeletal muscle as a model. Since, acetylcholine plays a significant role in contraction cycle and organophosphorus insecticides are found to inhibit the acetylcholinesterase, it is proposed to investigate the effect of malathion on contraction kinetics of the muscle.

Materials and Methods

Medium sized healthy frogs, *Rana hexadactyla* were double pithed and the gastrocnemius muscles from both the legs were excised with the least injury. The muscles were washed 4 times in amphibian Ringer's *medium*[9] and then allowed to stand in the same solution for 10 min for the recovery from the shock effects. Single muscle twitches were recorded before and after presoaking the muscles in Ringer solutions containing 0·001, 0·002, 0·003, 0·005 M malathion, as per the method of Uchida[10] *et al*, with slight modifications[11]. The muscle contractions were recorded on smoked paper pasted to a kymographic drum and the recordings were then fixed in turpentine-varnish mixture (1 : 3). The amplitude of contraction (or shortening) lengths, twitch duration, half contraction time (HCT) and half relaxation time (HRT) were calculated from the calibrated speed of the drum[12]. The average values of six observations were considered for the present study.

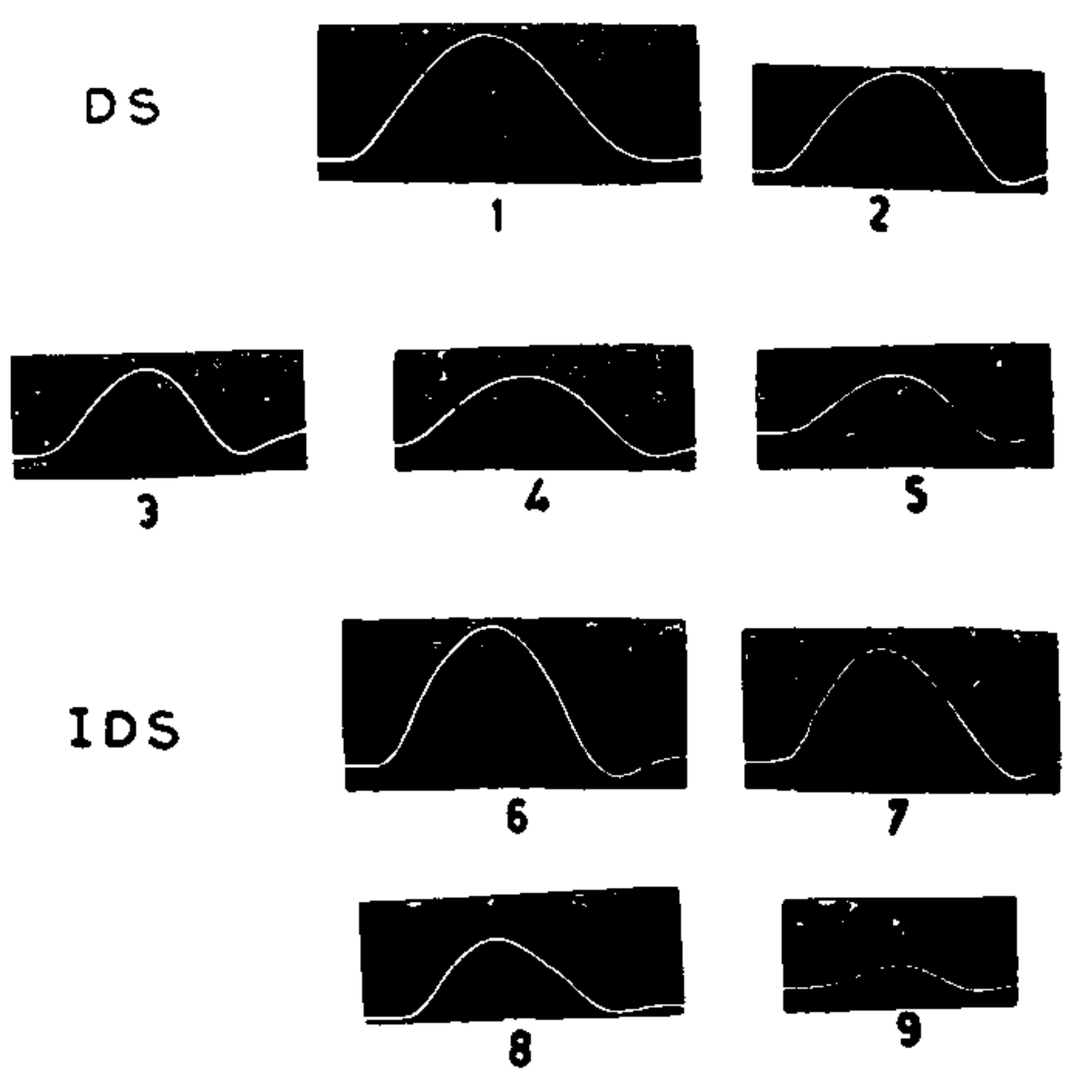

FIG. 1. Recordings of muscle contractions. Direct stimulation (DS)—(1) Normal Ringer, (2) 0·001 M Malathion, (3) 0·002 M Malathion, (4) 0·003 M Malathion, (5) 0·005 M Malathion. Indirect stimulation (IDS)—(6) Normal Ringer, (7) 0·001 M Malathion, (8) 0·002 M Malathion, (9) 0·003 M Malathion.

Results and Discussion

The amplitude of contraction, HCT, HRT and twitch duration in general showed a pronounced decrement both by direct (DS) and indirect stimulations (IDS) when the concentration of the insecticide varied from 0·001 to 0·005 M (Table I). The per cent decrement over control in the amplitude with DS were 17·2, 37·9, 44·8 and 55·2 for 0·001, 0·002, 0·003 and 0·005 M malathion respectively. During IDS, the per cent decrements over control were found to be 16·1, 45·2 and 83·9 for 0·001, 0·002 and 0·003 M respectively. At 0·005 M malathion, the response was found to be very low on IDS. These results clearly indicate that the neuromuscular junction is more susceptible to the insecticide than the muscle, as the decrease in the amplitude of the contraction was 55·2% on DS at 0·005 M and 83·9% on IDS

TABLE I

Amplitude, twitch duration, HCT, HRT of the muscles presoaked in different concentrations of malathion Ringer solutions

	Concentration of malathion in M	Direct stimulation (DS)					Indirect stimulation (IDS)				
		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.	0·000 (control)	33·99 ± 2·21	49·44 ± 2·13	1·45	2·90 ±0·12	166·86	29·72 ± 2·52	45·14 ± 2·92	1·52	3·10 ±0·17	149·72
2.	0·001	28·84 ± 1·17 P<0·001 (−15·15)	39·14 ± 2·82 P<0·001 (−20·23)	1·36 (− 6·70)	2·40 ±0·14 P<0·001 (−17·24)	135·96 (−18·50)	24·72 ± 2·55 P<0·001 (−16·82)	43·26 ± 3·17 NS (−4·16)	1·75 (+15·22)	2·60 ±0·14 P<0·001 (−16·13)	135·96 (− 9·19)
3.	0·002	22·66 ± 1·33 P<0·001 (−33·33)	37·96 ± 1·94 P<0·001 (−23·22)	1·67 (+15·16)	1·80 ±0·12 P<0·001 (−37·93)	121·24 (−27·44)	18·54 ± 1·92 P<0·001 (−37·62)	39·14 ± 2·12 P<0·01 (−13·62)	2·11 (+38·99)	1·70 ±0·12 P<0·001 (−45·16)	115·36 (−22·95)
4.	0·003	20·84 ± 1·52 P<0·001 (−38·69)	41·2 ± 3·12 P<0·01 (−16·67)	1·98 (+33·92)	1·60 ±0·22 P<0·001 (−44·83)	124·08 (−25·64)	14·42 ± 1·13 P<0·001 (−51·48)	26·78 ± 2·13 P<0·001 (−40·67)	1·86 (+22·27)	0·50 ±0·02 P<0·001 (−83·87)	82·40 (−44·96)
5.	0·005	19·21 ± 1·16 P<0·001 (−43·48)	32·08 ± 2·12 P<0·001 (−35·11)	1·67 (+14·81)	1·30 ±0·09 P<0·001 (−55·17)	102·58 (−38·52)	..	..	..	..	..

Values are mean, ± S.D. of six observations.

The values presented in parentheses are per cent deviation over control.

HCT : Half contraction time.

HRT : Half relaxation time.

at 0·003 M concentration of the insecticides. Thus the above contractions seemed invariably altered both with DS and IDS. Similar inhibitory patterns were observed in the HCT and HRT with DS and IDS when the muscle was presoaked in the organochloride insecticide (DDT)[11]. The extent of decrement in HCT was found to be more than in HRT on both DS and IDS, when the muscle was presoaked in Ringer solution with malathion. Similar inhibitory modulation on contractile kinetics were observed when the muscles were presoaked in normal and aestivated body fluids of *Pila*[13].

The greater inhibition of contractile kinetics of muscle in IDS as compared to DS, suggests greater involvement of this insecticide at neuromuscular junction. Present finding adds credence to the previous reports suggesting the irreversible inhibition of acetylcholinesterase and thereby changing the amounts of neurotransmitter, acetylcholine, with malathion[14,15]. In general it may be presumed that this organophosphorus insecticide alters the contractile potential of the muscle involving the neuromuscular junction, rather than the individual cellular response systems.

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1. Health, D. F., *Organophosphorus Poisons, Anticholinesterases and Related Compounds*, Pergamon Press, New York, 1961.
2. Karczmar, A. G., *Anticholinesterase Agents*, Pergamon Press, New York, 1961.
3. Koelle, G. B., *Cholinesterases and Anticholinesterase Agents*, Springer–Verlag, Berlin, 1963.
4. Murphy, S. D., *Proc. Soc. Exptl. Biol. Med.*, 1966, **123**, 392.
5. Fulkuto, T. R., *Bull. W.H.O.*, 1971, **44**, 31.
6. Zettler, J., Larry and Brady, E. U., *J. Econ. Entomol.*, 1972, **64**, 1034.
7. Kabeer Ahmed, I., Ramana Rao, K. V. and Swami, K. S., *Ind. J. Expt. Biol.*, 1978, **16**, 58.
8. Murphy, S. D., Lauwerys, R. L. and Cheever, K. L., *Toxicol. Appl. Pharmacol.*, 1968, **12**, 22.
9. Cavanaugh, G. M., *Formulae and Methods*, Marine Biological Laboratory, Woodshole, Mass., 1956, **4**.
10. Uchida, M., Naka, Y., Irie, Y., Fujita, T. and Nakojima, M., *Pestic. Biochem. and Physiol.*, 1974, **4**, 451.
11. Rajendra, W., Sreeramulu Chetty, C., Anaruya, R., Indira, K. and Swami, K. S., Unpublished Data, 1979.
12. Venkateswarlu, D. and Sasirababu, K., *Ind. J. Expt. Biol.*, 1976, **14**, 429.
13. Indira, K, Swami, K. S., Sreeramulu Chetty, C. and Rajendra, W., *Ind. J. Expt. Biol.*, 1976, **16**, 261.
14. Karczmar, A. G., Nishi, S. and Blaber, L. C., *Acta Vitaminol. Enzymol.*, 1970, **24**, 131.
15. O'Brein, R. D., *Toxic Phosphorus Esters*, Academic Press, New York, 1960.

EFFECT OF NUCLEAR POLYHEDROSIS VIRUS OF THE ARMYWORM *MYTHIMNA (PSEUDALETIA) SEPARATA* ON THE TASAR SILKWORM *ANTHERAEA MYLITTA*

NUCLEAR Polyhedrosis Virus (NPV) of the armyworm *Mythimna (Pseudaletia) separata* is successfully used to control its host which is a serious agricultural pest[1]. However, large scale use of NPV needs safety tests on beneficial insect like tasar silkworm *Antheraea mylitta.* Since the effect of NPV of the armyworm *M. (P.) separata* has not been investigated the present experiments were conducted.

Tasar silkworm larvae reared on *Terminalia tomentosa* were treated with following concentrations of virus higher than those required to infect the army worm: 10×10^5 Polyhedral Inclusion Bodies/Larva, 10×10^6 PIBs/L, 10×10^7 PIBs/L and 10×10^8 PIBs/L. While in oral (Experiment I) and topical (Experiment II) treatments, 50 fifth instar larvae were used in 4 replications, during intrahemocoelic injection (Experiment III) treatment 40 fifth instar larvae were replicated 5 times. In all the three experiments, controls generally received distilled water. However, in the III experiment another set of control received alkaline solution ($NaCl + Na_2CO_3$) to free the viral rods from PIBs.

Observations were made daily to determine the larval death due to NPV and other causes, and also on per cent pupation.

Results obtained from the three experiments could be summarised as follows. The treated larvae showed neither any signs and symptoms, nor mortality due to polyhedrosis. Pupation rate was 60–84%. Further, the treated larvae did not significantly differ from the controls in their cacoon formation. Hence, it appears that NPV of the armyworm is non-infective to *A. mylitta.* In experiment I (P.O. treatment) we also attempted to note the fate of the PIBs in the tasar silkworm bodies by examining periodically the gut and faecal matter. Though PIBs could be found in the gut lumen but not in the faecal matter of the treated larvae, after $3\frac{1}{2}$ h from the time of treatment, they were not detected either in the gut or in faecal matter after 24 h. The findings, therefore, suggest that though the protein coat of PIBs is dissolved in the gut, the virus is non-infective to the tasar silkworm *A. mylitta.* In topical application (Experiment II) when the larval body was gently scraped and observed for the PIBs, we could find PIBs after 15 days of treatment This finding