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# *In vitro* Effect of Some Anthelmintics on the Motility of *Gigantocotyle explanatum*

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**Abstract**

The effect of different anthelmintics on the *in vitro* motility of *Gigantocotyle explanatum* have been investigated by means of an isometric transducer system. Mebendazole induces irreversible spastic paralysis, whereas Fenbendazole causes irreversible disturbed activity. Metrifonate produce rapid decrease in muscle tone leading to flaccid paralysis. Oxyclozanide induce immediate suppression of motility with increased muscle tone leading to irreversible spastic paralysis. Further, quantitative differences are noticed in each drug to produce particular effect. Possible explanation for these effects on the worm motility are discussed.

**Key words:** *Gigantocotyle explanatum,* isometric transducer, motility, anthelmintics, *in vitro*

## Introduction

Paramphistomes are generally characterized by the well developed and highly muscular acetabulum which provides firm attachment to the host tissue, and prevents the dislodgement from the muscular contraction or with secretions of host. Therefore, any disturbance in the parasite's neuromuscular co-ordination could be used as an effective target for control of the parasites. Hence motility of the parasites has been used as an effective parameter for the evaluation of drug efficacy by many workers (Mansour, 1964; Saz and Bueding, 1966; Woolhouse, 1979; Terada, *et al.,* 1982a, b; Fairweather *et al.*, 1984). Recently Boray (1986) reviewed the efficacy of various anthelmintics in amphistomes.

The present study aims to investigate the pharmacological effects of some anthelmintics on *in vitro* motility of *G. explanatum* by using isometric transducer, which is more sensitive technique for monitoring the *in vitro* motility than the previously used experimental set ups described by Fairweather, *et al.* (1983).

## Materials and Methods

Mature *Gigantocotyle explanatum* were collected from the bile ducts of water buffaloes *Bubalus bubalis* slaughtered at the local abattoir. After isolation from liver tissue the worms were kept in Hank's Balanced Salt Solution (HBSS) premaintained at $37 \pm 2°C$.

For the qualitative analysis of the changes in the worm motility due to drug action, the experiment was designed to record the motility with the help of an isometric transducer. The apparatus used was designed as described by Fairweather *et al.* (1983), with some modifications. The flow of medium in the inner parasite vessel was controlled by a peristaltic pump (LKB, Sweden). The worm was suspended vertically within the vessel and acetabulum fixed with the help of a heart clip. The anterior end of the fluke was attached to the isometric force displacement transducer (Type D, Palmer Bioscience, Washington), which in turn was connected through a preamplifier and strain gauge coupler (Type CD10, and Type FC 117 respectively, Palmer Bioscience, Washington) to a potentiometric pen recorder (Hindustan Powertronix Incorporation, India), operating at a speed of 0.2 cm/sec. The worm suspended in the vessel was allowed to recover for a period of 30 min in a relaxed condition, before being placed under tension. First the normal motility was recorded in a drug free HBSS and thereafter the medium was replaced slowly and gently with the warm HBSS containing drugs in required concentrations as described by Fairweather *et al.*

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(1984). The changes produced by the drugs in worm motility were recorded and the post washed recordings were also made in order to confirm whether the drug effect was reversible or irreversible.

The drugs tested in the present study were mebendazole (Mbz), fenbendazole (Fbz), metrifonate (Mf) and oxyclozanide (Oxy), and the concentrations used were the same as used for the biochemical and topographical effects (Ahmad, 1984; Ahmad *et al.*, 1987). Mbz and Fbz were dissolved in dimethyl sulfoxide (DMSO) in a final concentration of 0.1% in HBSS. Oxy was dissolved in 3% ethanol whereas Mf was prepared fresh in HBSS. For control experiments, it was found that DMSO in 0.1% and ethanol in 3% concentration had no effect on the normal movement of the worms. For each drug, at least eight recordings were made and a separate worm was used for each recording.

## Results

Normal *in vitro* motility recordings of the worm reveal that *G. explanatum* is a highly active and motile parasite, showing prominent muscular contraction and relaxation movements in HBSS. The normal movement of each worm is peculiar to individual recordings. However, it is typical that the movement of each worm is rhythmical in nature, with a regular burst of contraction and a short period of basal activity. The burst of activity is followed by a less active lull and this is almost a regular sequence. The frequency of the burst phase is more as compared to the lull phase. The amplitude of contractions in the burst phase and that of the basal activity remains somewhat constant. The normal movement in terms of frequency and amplitude of contractions and relaxations is easily maintained for a period of 6 to 8 h. The "normal" pattern of activity is used to compare with the "drug-affected" movements, recorded after the drug addition (indicated by a bold arrow) in each case.

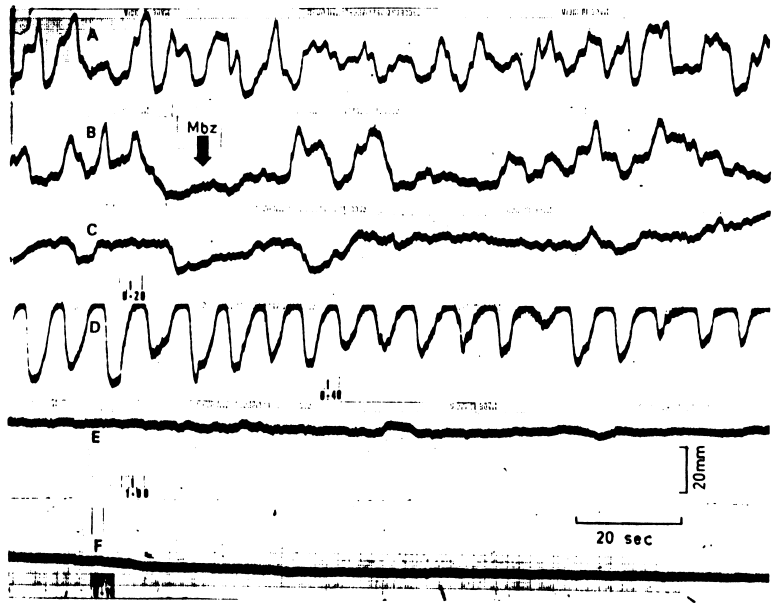


Fig. 1. Effect of Mebendazole ($3.3 \times 10^{-6}$M) on *in vitro* motility of *G. explanatum*. Broad arrow indicates addition of drug and numbers indicate time in hours.
A: Normal activity.
B–E: Activity in presence of drug.
F: Activity after 0.1h post-washing.

Mebendazole at a concentration of $3.3 \times 10^{-6}$M causes a gradual change in the normal movement (Fig. 1A) producing disturbance in the rhythmic contraction and relaxation (Fig. 1B). Gradually after 20 minutes, the frequency of the bursts and lulls and their amplitude is also disturbed, while at the same time there is an increase in the muscle tone (Fig. 1C). After 40 minutes, the activity is drastically disturbed, with increased frequency of bursts and lulls (Fig. 1D). The sustained tonic contraction of the musculature with slight indication of intermittent activity was observed after one hour (Fig. 1E). The post-washing recording reveal that the muscle tone decreased gradually but the effect of Mbz is irreversible (Fig. 1F).

Soon after the addition of fenbendazole ($1 \times 10^{-5}$M) there is a gradual disturbance in the bursts and lulls of the worm's activity (Fig. 2B). Slowly, the burst activity increases, with an increase in the basal activity, and the frequency of movement increases with a decrease in amplitude (Fig. 2C and 2D). The post-washed recordings show that the muscle tone is decreased but the activity is still there, although with a drastic change (Fig. 2E). Thus, febendazole effect is irreversible but the worm remains active with disturbed activity.

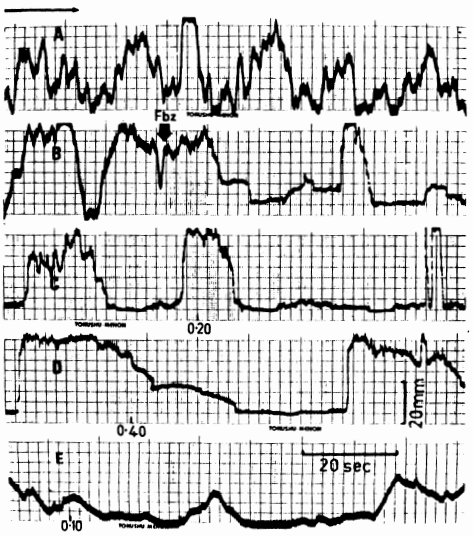


Fig. 2. Effect of Fenbendazole ($1 \times 10^{-5}$M) on *in vitro motility of G. explanatum*. Broad arrow indicates addition of drug and numbers indicate time in hours.
A: Normal activity.
B–D: Activity in presence of drug.
E: Activity after 0.10h post-washing.

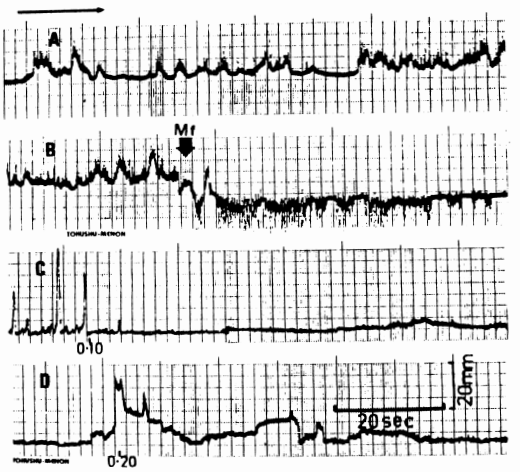


Fig. 3. Effect of Metrifonate ($19.4 \times 10^{-5}$M) on *in vitro* motility of *G. explanatum*. Broad arrow indicates addition of drug and numbers indicate time in hours.
A: Normal activity.
B and C: Activity in presence of drug.
D: Activity after 0.20h post-washing.

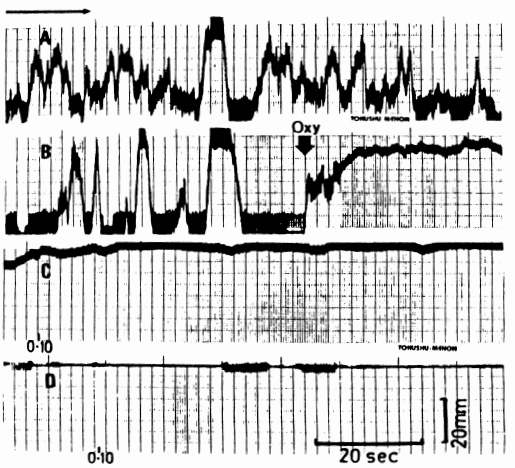


Fig. 4. Effect of Oxyclozanide ($1 \times 10^{-5}$M) on *in vitro* motility of *G. explanatum*. Broad arrow indicates the addition of drug and numbers indicate time in hours.
A: Normal activity.
B and C: Activity in presence of drug.
D: Activity after 0.10h post-washing.

Metrifonate ($19.4 \times 10^{-5}$M) induces a rapid decrease in muscle tone and the activity is lost within 10 minutes (Fig. 3B). Muscle tone is decreased and the basal activity is reduced drastically, and it appears as if a flaccid paralysis has been reached (Fig. 3C). The drug is fairly quick in action, although after washing, the worm regains some activity, with disturbed burst and lulls (Fig. 3D).

Oxyclozanide ($1 \times 10^{-5}$M) causes a fairly immediate suppression of motility, accompanied by an increase in muscle tone to a spastic paralysis (Fig. 4B, C). The effect is irreversible, since the worm muscle tone remains increased even after washing (Fig. 4D).

## Discussion

Generally, *in vivo* testing of the efficacy of various compounds against parasites is very expensive and time consuming. In comparison, the present *in vitro* system is relatively simple, quick and inexpensive.

It is evident that the benzimidazole compounds affect the neuromuscular system of the amphistome, although mebendazole induces a variety of biochemical actions in parasites, including impairment of glucose uptake, glycogen depletion, changes in adenine nucleotide levels, and changes in the enzymes of glycolysis (Van Den Bossche, 1976; Behm and Bryant, 1979; Ahmad and Nizami, 1987). Fenbendazole, on the other hand, inhibits the fumarate reductase (FR) system (Prichard *et al.*, 1978) and such inhibition not only prevents succinate formation, but also the production of ATP and regeneration of NAD, and this disrupts the terminal electron transport in parasitic helminths (Fioravanti & Saz, 1980). Fenbendazole, like mebendazole, is also reported to cause depletion of glycogen levels and inhibition of glucose absorption in helminths (McCracken and Taylor, 1983; Duwel, 1977). It is possible that the benzimidazole compounds under study impaired the generation and utilization of energy reserves, followed by loss of neuromuscular co-ordination. Mbz gradually leads to complete loss of activity which is irreversible, whereas Fbz is more rapid in action, leading to disturbed bursts and lulls. This may be due to the fact that a single drug may have a variety of mode of actions, so it is difficult to say whether a particular change is due to a single effect or because of a combination of effects. It is most likely that the primary effect of these benzimidazole compounds is to induce metabolic disorder and topographical damage and the secondary effect is in neuromuscular co-ordination in *G. explanatum,* since Ahmad *et al.* (1987) have reported the sloughing of the tegumental surface due to benzimidazole in this parasite.

The effect of metrifonate on the motility of *G. explanatum* is fairly rapid and the muscle tone is decreased, leading to a flaccid condition. A similar effect has been noticed in *S. mansoni* by Semeyn *et al.* (1982) and Mellin *et al.* (1983). Effect of metrifonate in *G. explanatum* is irreversible, since the worm activity is not regained and the frequency, amplitude and rhythmicity of bursts and lulls do not reach normal levels. It appears that, unlike the benzimidazole compounds, metrifonate's effect is neuromuscular in nature. Thus, metrifonate is known to inhibit acetylcholinesterase activity in helminth parasites (Reiner *et al.* 1979; Reiner, 1981; Gunn and Probert, 1981). Hence Mf inhibits acetylcholinesterase activity and blocks neurotransmission, which in turn leads to a flaccid paralysis.

Oxyclozanide in the present study caused irreversible spastic paralysis in a very short period. Fairweather, *et al.* (1984) also reported spastic paralysis due to oxyclozanide in *F. hepatica.* Oxy is also known to uncouple oxidative phosphorylation in helminths, thus blocking ATP synthesis and energy production (Corbett and Goose, 1971; Veenendaal and De Waal, 1974). Therefore, it is likely that the spastic paralysis due to Oxy, may be a combination of neuromuscular and metabolic effects.

Preliminary studies, on detachment of *G. explanatum* in the presence of the anthelmintics under study revealed that these drugs induce worm detachment from the host tissue under *in vitro* conditions (Ahmad, 1984). Similar results have also been reported in *F. hepatica* due to Mbz and rafoxanide under *in vivo* conditions (Cornish,

*et al.*, 1977; Rahman *et al.*, 1977; Chevis, 1980).

It is concluded from the present study that, in addition to known biochemical effects, these drugs also interfere with neuromuscular activity, as revealed by the differences in the motility recordings. Further, the present device provides an opportunity for primary screening of drugs under *in vitro* conditions for long periods and provide basic information for subsequent *in vivo* trials, as suggested by other workers (Terada *et al.*, 1982a, b; Fairweather *et al.*, 1984).

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