Monoamine oxidase in amphistomes and its role in worm motility

S.M.A. Abidi* and W.A. Nizami

Section of Parasitology, Department of Zoology,
Aligarh Muslim University, Aligarh-202 002, India

Abstract

The quantitative assay of mitochondrial monoamine oxidase (MAO) activity revealed a higher enzyme level in *Explanatum explanatum* than *Gastrothylax crumenifer*. The specific MAO inhibitors, chlorgyline, pargyline, deprenyl and nialamide produced different degrees of interspecific inhibition. The differential effects on enzyme activity of chlorgyline and deprenyl suggests the possible existence of polymorphic forms of the enzyme, MAO-A and MAO-B, in amphistomes. These specific inhibitors also had a differential influence on the *in vitro* motility of amphistomes, further indicating the involvement of different forms of MAO in the oxidative deamination of biogenic monoamines which might be partly responsible for neuromuscular coordination in amphistomes. The experimental procedures used in this study could be conveniently used for quick screening and evaluation of some of the qualitative effects of anthelmintic drugs under *in vitro* conditions.

Introduction

The oxidative deamination of biogenic monoamines is actively brought about by the enzyme monoamine oxidase, which thereby acts as a major scavenger of monoamines released at the neuronal junctions as well as non-neurotransmitter monoamines present in intestine and other peripheral tissues (Agarwal *et al*., 1990). The quantitative and qualitative differences in the distribution of biogenic amines which might be involved in the neuromuscular activities, behavioural coordination and metabolic regulation, and also an active MAO, have been observed in a number of helminth species (Mansour, 1962; Nimmo-Smith & Raison, 1968; Bennett & Bueding, 1971; Chou *et al*., 1972; Hariri, 1974; Moreno & Barrett, 1979; Mishra *et al*., 1983; Agarwal *et al*., 1985; Smart, 1988). However, very limited information is available about the role of MAO in the neuromuscular coordination of helminths in general and almost nothing is known in the case of amphistomes.

In the present communication, preliminary studies have been carried out on monoamine oxidase in two amphistome species infecting the Indian water buffalo, *Bubalus bubalis*, and its involvement in neuromuscular coordination has been investigated.

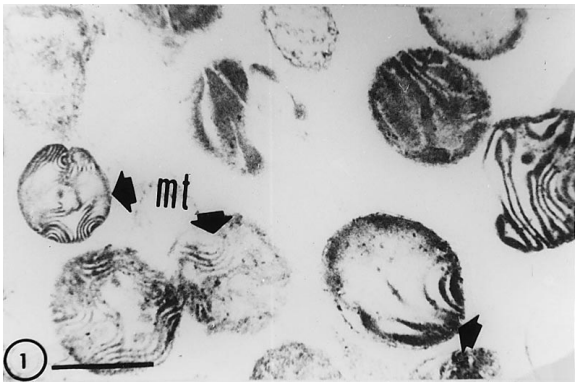
Materials and methods

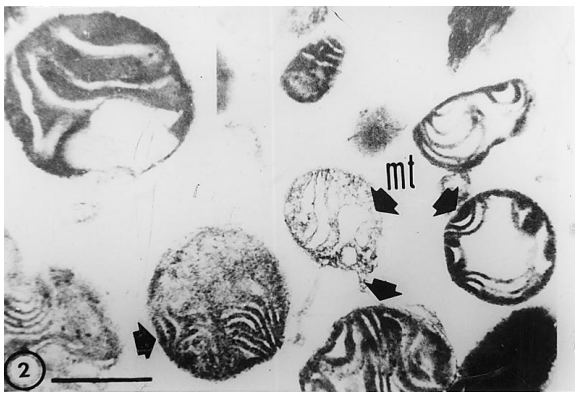
Mature worms of *Explanatum explanatum* and *Gastrothylax crumenifer* were collected from the buffalo liver and rumen, respectively. The worms were thoroughly washed in Hanks' medium without glucose, pH 7.4, premaintained at 37 ± 1°C and processed separately. For a quantitative assay the worms were homogenized at 4°C in a glass teflon tissue grinder fitted with a motor driven pestle, using 0.05 M tris-HCl, pH 6.8, containing 0.25 M sucrose, 100 mM KCl, 1 mM EDTA, 1 mM $MgCl_2$, 1 mM ATP and 0.5% bovine serum albumin (BSA). The mitochondria were isolated by differential centrifugation as described by Podesta *et al*. (1986) and the purity was checked through standard transmission electron microscopy (TEM) procedures (figs 1 and 2).

Before taking the samples for estimations, the mitochondrial pellet was suspended in a known volume of tris-HCl buffer, pH 7.4, and sonicated at 0°C for 30 s on an ultrasonic disintegrator (Ralsonics, India) using a probe of 5 mm diameter. The homogeneous suspension was used for the enzyme assay and the dilution factor was taken into account for calculations.

*Fax: +91 571 408069
E-mail: zyr015@amu.up.nic.in

https://doi.org/10.1017/S0022149X00701039 Published online by Cambridge University Press





Figs 1–2. Transmission electron micrographs showing mitochondria isolated from the liver and rumen amphistomes *Explanatum explanatum* (1) and *Gastrothylax crumenifer* (2) respectively. Distinct cristae can be seen. Scale bar = 0.25 μm.

Monoamine oxidase [E.C. 1.4.3.4] assay

Enzyme activity was determined in the mitochondrial fractions as described by Weissbach *et al*. (1960) and Catravas *et al*. (1977) with some minor modifications. The enzyme assay mixture of 3 ml final volume contained 0.05 M tris-HCl, pH 7.4, 0.22 mM kynuramine dihydrobromide, 0.08 mM $MgCl_2$, and sonicated mitochondrial pellet in the assay buffer. The test tubes containing the reaction mixture were incubated at 37°C for 30 min and the reaction was stopped by the addition of 0.5 M NaOH and 10% zinc sulphate. The mixture was heated in a boiling water bath for 5 min and then centrifuged at 10,000 × *g* for 10 min. The concentration of the reaction product 4-hydroxyquinoline was determined in the supernatant spectrophotometrically by measuring the absorbance at 330 nm. A blank was prepared by replacing the substrate with distilled water. Values of the unknown samples were calculated from the standard curve prepared using different concentrations of 4-hydroxyquinoline.

The specific enzyme activity is expressed as μmoles 4-hydroxyquinoline produced per mg protein per 30 min at 37°C.

Effect of inhibitors

To examine the effects of specific MAO inhibitors, nialamide, pargyline, chlorgyline and deprenyl at a final concentration of 5×10^{-4} M, 5×10^{-5} M, 2×10^{-4} M and 2×10^{-4} M, respectively were added to the assay mixture and changes in absorbance recorded.

Protein was estimated by the dye-binding method of Spector (1978), using bovine serum albumin as standard.

All results were subjected to statistical analysis and the level of significance was determined using the student's t-test as described by Sokal & Rohlf (1981).

In vitro *motility*

The specific effects of MAO inhibitors on the motility of the two amphistomes were screened, as possible means of providing indirect evidence for the existence of an aminergic pathway, involving monoamines for neuromuscular coordination in amphistomes. The changes in worm motility due to the action of MAO inhibitors were recorded using an isometric force transducer assembly, as described by Ahmad & Nizami (1991). The isometric force displacement transducer (Type D, Palmer Bioscience, Washington) was connected through a preamplifier and strain gauge coupler (Type CD 10 and Type FC 117, respectively, Palmer Bioscience, Washington) to a potentiometric pen recorder (Hindustan Powertronix Inc., India), operating at a speed of 0.2 cm s^{-1}. Normal worm motility was initially checked for 2 h and thereafter in each experiment it was recorded for 15 min in inhibitor-free Hanks' medium. The medium was then slowly replaced with Hanks' containing inhibitors by a peristaltic pump (LKB, Sweden), maintaining the following

Table 1. The effect of inhibitors on the mitochondrial monoamine oxidase activity* in *Explanatum explanatum* and *Gastrothylax crumenifer*.

Parasite	Normal activity	Inhibitors			
		Nialamide	Pargyline	Chlorgyline	Deprenyl
E. explanatum	303.98 ± 4.16	277.41 ± 5.19 (8.74)	259.96 ± 11.58 (14.48)	276.58 ± 9.67 (9.01)	296.51 ± 5.10 (2.46)
G. crumenifer	293.43 ± 6.41	281.11 ± 10.89 (4.19)	293.13 ± 12.34 (0.10)	281.51 ± 7.86 (4.06)	287.25 ± 9.38 (2.11)

* μmoles hydroxyquinoline produced per mg protein per 30 min at 37°C ± SEM.
Figures in parentheses indicate percent inhibition.
In all the tests at least three replicates were performed.

https://doi.org/10.1017/S0022149X00701039 Published online by Cambridge University Press

concentrations: nialamide 50×10^{-4} M ml^{-1}, pargyline 5×10^{-5} M ml^{-1}, chlorgyline 2×10^{-4} M ml^{-1} and deprenyl 2×10^{-4} M ml^{-1}. Changes in worm motility were recorded for different time periods depending upon the responses of worms to the inhibitors. Thereafter, worms were washed with normal Hanks' and post-wash recordings were also made in order to confirm whether or not the inhibitor effect was reversible.

Results

Physiologically active amines are known to be widely distributed in parasitic helminths and may act as neurotransmitters. Since they would produce toxicity if retained following secretion, the amines are detoxified by various enzymes including monoamine oxidase. The level of mitochondrial MAO, as determined in the present study, was found to be slightly higher in the liver amphistome, *E. explanatum* as compared to the rumen parasite, *G. crumenifer* (table 1). However, this difference was statistically insignificant ($P > 0.05$). When specific inhibitors of MAO, nialamide, pargyline, chlorgyline and deprenyl were added to the assay mixture, some variable inhibitory effects were observed (table 1). Only pargyline produced relatively more inhibition of enzyme activity, followed by chlorgyline and nialamide in *E. explanatum*, whereas in *G. crumenifer* mitochondrial MAO was found to be insensitive to the concentrations of inhibitors used for the quantitative assay.

For the investigation of the qualitative influence of inhibitors, the effect was monitored on worm motility (figs 3 and 4). The effect of pargyline on the motility of the two amphistome species was found to be quite different from each other. As in the liver amphistome, *E. explanatum*, the contraction phase was prolonged with a gradual loss of rhythmical activity, leading to spastic paralysis which remained even during post-wash recordings (fig. 3a), whereas, contrary to this, the frequency of contractions in the rumen amphistome *G. crumenifer* initially increased during the first 15 min period but then decreased resulting in a flaccid condition with intermittent spikes without any appreciable shift in the amplitude (fig. 3b). Chlorgyline produced irreversible spastic paralysis in both genera. However, the effect was more pronounced in the liver amphistome *E. explanatum* (fig. 3c,d). Deprenyl did not alter the motility of *E. explanatum* but in *G. crumenifer*, the frequency of contraction was slightly increased during the first 10 min, following which the amphistome underwent a spastic condition with occasional spikes (fig. 4a,b). Nialamide prolonged the phase of contraction in *E. explanatum* but the motility was not completely disrupted, while in *G.*

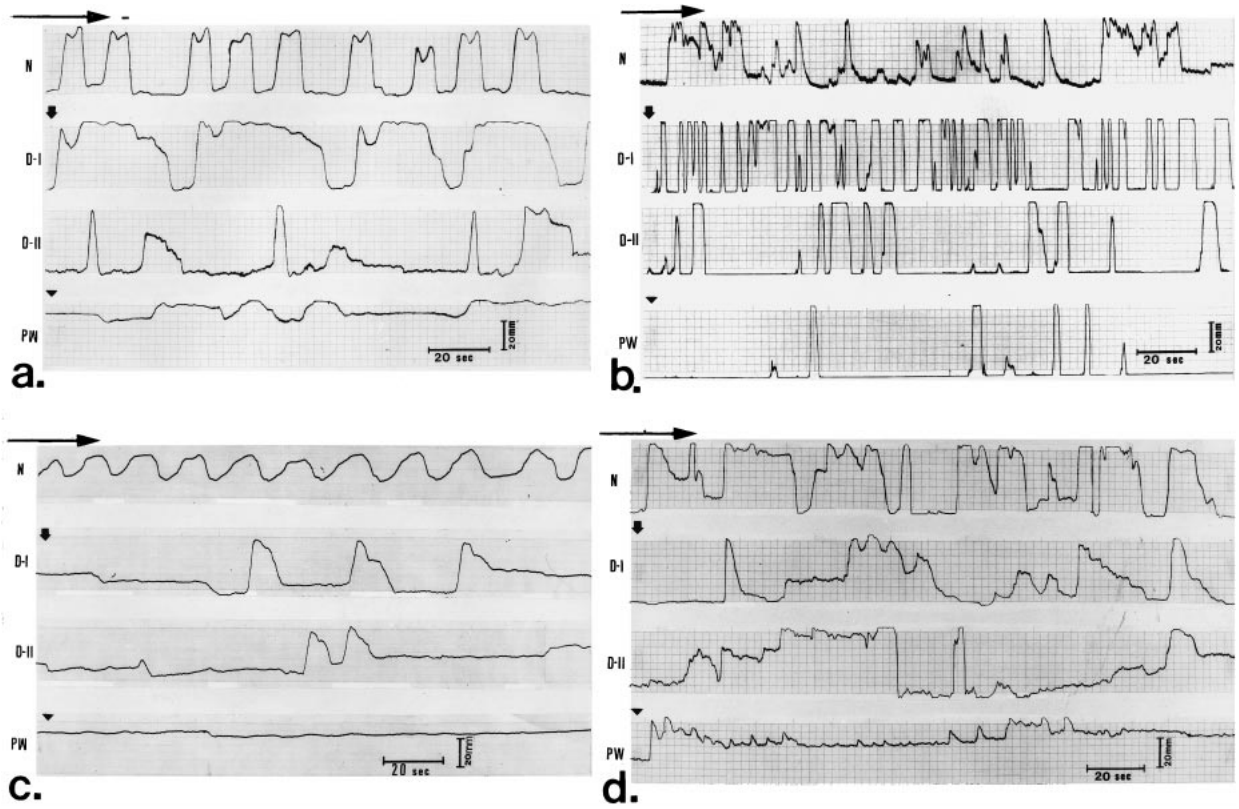


Fig. 3. The effects of monoamine oxidase inhibitors, pargyline (a and b) and chlorgyline (c and d) on the *in vitro* motility of *Explanatum explanatum* (a and c) and *Gastrothylax crumenifer* (b and d). N, normal worm motility; D-I and D-II, worm motility in presence of inhibitors; PW, post-wash recordings; horizontal arrow, direction of worm motility recording; arrow, point of inhibitor addition; arrow head, start of post-wash recordings.

https://doi.org/10.1017/S0022149X00701039 Published online by Cambridge University Press

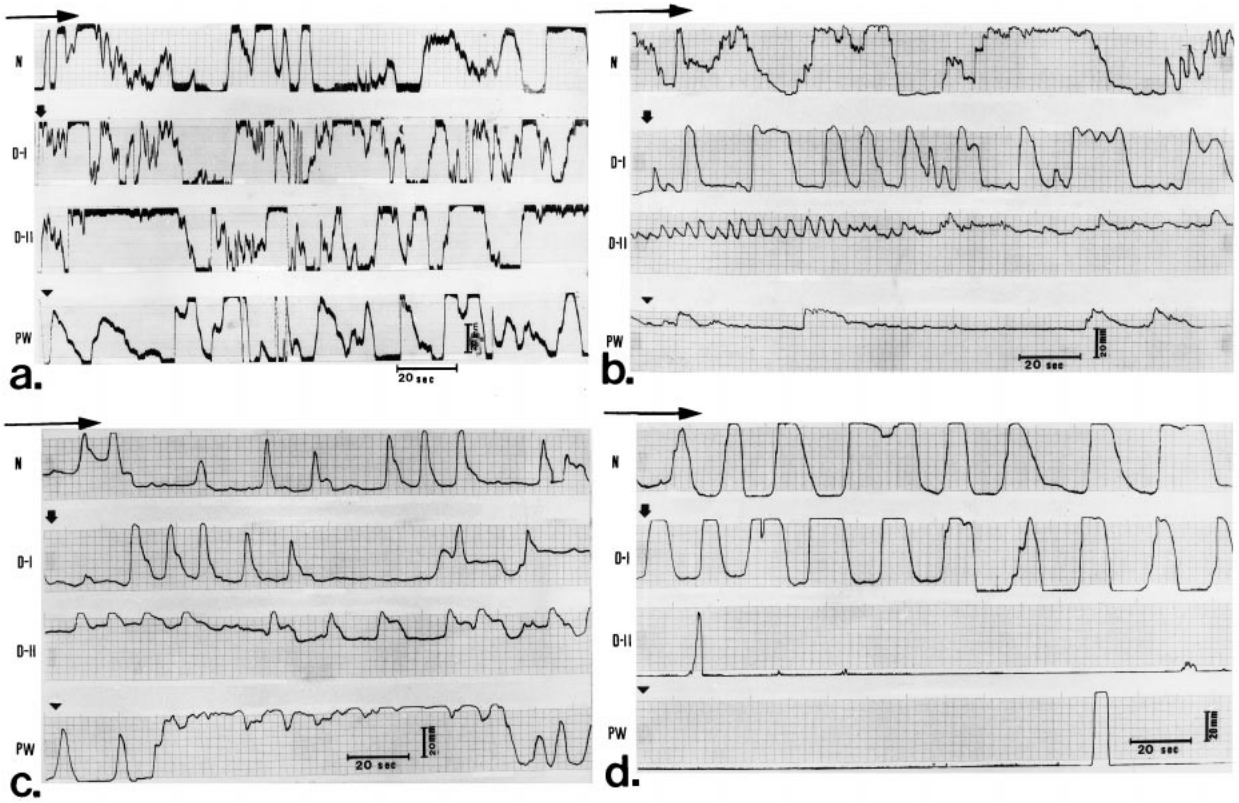


Fig. 4. The effect of monoamine oxidase inhibitors deprenyl (a and b) and nialamide (c and d) on the *in vitro* motility of *Explanatum explanatum* (a and c) and *Gastrothylax crumenifer* (b and d). N, normal worm motility; D-I and D-II, worm motility in presence of inhibitors; PW, post-wash recordings; horizontal arrow, direction of worm motility recording; arrow, point of inhibitor addition; arrow head, start of post-wash recordings.

crumenifer it produced a flaccid paralysis since worms did not attain normal activity even after removal of the inhibitor and subsequent washing (fig. 4c,d). Considering the mode of paralysis, the effects of pargyline and nialamide were similar in the case of *G. crumenifer* where the worms undergo a flaccid state.

Discussion

The catalytic activity of monoamine oxidase is an important step in the degradation of biogenic amines. Mitochondrial MAO activity in the liver amphistome, *E. explanatum*, was found to be higher than that in the rumen amphistome, *G. crumenifer*, thus showing an intergeneric variation in enzyme levels.

The differential responses of MAO to nialamide, pargyline, chlorgyline and deprenyl reflect differences in the parasite enzymes, adapted, possibly to their respective microenvironments, since parasite metabolism is greatly influenced by habitat (Von Brand, 1979; Barrett, 1981). The helminths under study occupy quite different habitats within the host. Thus, *E. explanatum* in the liver occupies a nutritionally rich habitat and remains in a more or less constant environment, and the MAO activity was found to be sensitive to the specific inhibitors used in the present study. On the other hand, *G. crumenifer* in the rumen occupies a habitat that is continuously exposed to ionic and osmotic fluctuations resulting from the dietary habits of the host, and the pressure of microbes in the rumen, and results reveal a narrow range of inhibitory responses to the various MAO inhibitors, indicating an adaptive tolerance by the rumen parasites. It has been suggested, for gastrointestinal helminths in particular, that the main function of parasite MAO may be to protect parasites from exogenous amines present in the host gut (Mishra *et al*., 1978; Moreno & Barrett, 1979).

Among various inhibitors used in the present study, chlorgyline (Johnston, 1968) and deprenyl (Knoll *et al*., 1965) play an important role in describing and distinguishing two main types of mitochondrial MAO, referred to as type A (serotonin oxidase) and type B (B-phenylethylamine oxidase), respectively. In this respect, the differential inhibition of MAO activity by chlorgyline and deprenyl suggests that both A and B types of MAO are present in the parasites under study. Chlorgyline was found to exert more inhibition in *E. explanatum*, compared with *G. crumenifer*. However, quantitative inhibition of MAO-B was more or less the same in the two amphistomes in response to deprenyl, which specifically inhibits MAO-B, and this polymorphic form appeared to be predominantly involved in the neuromuscular coordination of *G. crumenifer*. On the other hand, MAO-A could be

https://doi.org/10.1017/S0022149X00701039 Published online by Cambridge University Press

mainly involved in the motility of *E. explanatum*, as shown from the responses of these worms to chlorgyline. It has been suggested that inhibitors of MAO potentiate the effects of noradrenaline and other amines by blocking their degradation in the cytosol by the MAO present in the outer mitochondrial membrane (McGilvery & Goldstein, 1979). Using a Clark-type oxygen electrode, Moreno & Barrett (1979) observed 18, 30 and 45% inhibition of MAO in *Hymenolepis diminuta* when incubated in the presence of iproniazed (300 μm) nialamide and pargyline, respectively. Mishra *et al*. (1985) also reported 65, 72, 93 and 96% enzyme inhibition in *Ascaridia galli* by iproniazed, nialamide, pargyline and trans-PcP, respectively. Using a different substrate, Nimmo-Smith & Raison (1968) suggested the existence of two forms of MAO in schistosomes. Therefore, the differential response of the enzyme to the inhibitors under study could be due not only to the origin of the enzyme but also to the intergeneric variation in the isozymes, reflecting an adaptation in response to different metabolic requirements during the course of the life cycle of the parasite.

Although MAO activity was differentially inhibited by various specific inhibitors, it is necessary to investigate the I_{50} (50% inhibition) of different specific inhibitors in order to characterize the enzyme molecules of different helminths. In the present investigation, the inhibitors were directly added to the assay mixture, such that the degree of MAO inhibition was low compared with the previous studies where enzyme preparation was preincubated with the inhibitors. In fact, the present procedure reflects a more immediate response of MAO to the inhibitors in question.

Biogenic monoamines are known to be involved in the neuromuscular coordination of helminths, and once their function as a neurotransmitter is accomplished, they are degraded by enzymes, such as MAO. An inhibition of MAO would lead to their accumulation, thereby causing toxicity which could disrupt normal movements of the worms. The modulation of worm motility by the specific inhibitors of MAO provides indirect evidence for the occurrence of an aminergic pathway and the probable role of monoamines in the neuromuscular coordination of amphistomes, *E. explanatum* and *G. crumenifer*. The effects of drugs on the motility of parasitic helminths have been tested as one of the useful parameters for the investigation of anthelmintics (Terrada *et al*., 1982).

The effect of nialamide on the motility of *E. explanatum* was recorded in the form of changes in the amplitude and frequencies with a prolonged phase of contraction; the worms did not restore normal activity, whereas in *G. crumenifer* nialamide produced a flaccid condition which was irreversible. Pargyline produced an irreversible, spastic condition in *E. explanatum* while in *G. crumenifer* it produced a reversible flaccid condition. The characteristic motility response to chlorgyline and deprenyl suggests that MAO-A catabolized biogenic monoamines are involved in the neuromuscular coordination of amphistomes, whereas MAO-B could be involved in the catabolism of monoamines only in *G. crumenifer*, as deprenyl did not produce any noticeable change in the motility of *E. explanatum*. These results therefore indicate the existence of polymorphic forms of MAO in the two amphistomes under study. The effect of different drugs or aminergic agents on the *in vitro* motility of *Fasciola hepatica* has been investigated by Holmes & Fairweather (1984). Serotonin is capable of exerting a power influence on the various aspects of carbohydrate metabolism in *F. hepatica* (Mansour, 1979), but its role as a neurotransmitter in *F. hepatica* could not be resolved (Holmes, 1984), although it is widely distributed in the cerebral ganglia, longitudinal cords, commissures and in sensory structures of various flatworm classes (Halton & Gustafsson, 1996).

It is concluded that biogenic monoamines are involved in neuromuscular coordination in amphistomes and probably also regulate other activities in the worms as in most invertebrates. Thus, in concert with neuropeptides they may also control a number of ecophysiological processes which regulate the biological clock in these organisms. Furthermore, worm motility could be employed as a useful parameter for screening the effects of metabolic inhibitors.

Acknowledgements

This communication is dedicated to the profound memories of the late Professor W.A. Nizami who passed away in October 1998. The author is thankful to the Chairman, Department of Zoology, for providing the necessary laboratory facilities. Financial assistance from the Indian Council of Agricultural Research, New Delhi; travel and instrument grants from the Wellcome Trust and the British Council, UK and assistance by Dr G. Ahmad are gratefully acknowledged.

References

Agarwal, A., Mishra, A., Mishra, S.K. & Ghatak, S. (1985) Monoamine oxidase in *Nippostrongylus brasiliensis*. *Indian Journal of Parasitology* **9,** 221–224.

Agarwal, A., Shukla, O.P., Ghatak, S.N. & Tekwani, B.L. (1990) Biogenic amines, metabolites and monoamine oxidase in the filarial worm *Setaria cervi*. *International Journal for Parasitology* **20,** 873–881.

Ahmad, M. & Nizami, W.A. (1991) *In vitro* effect of some anthelmintics on the motility of *Gigantocotyle explanatum*. *Japanese Journal of Parasitology* **39,** 529–534.

Barrett, J. (1981) *Biochemistry of parasitic helminths*. London and Basingstoke, Macmillan Publishers, Ltd.

Bennett, J. & Bueding, E. (1971) Localization of biogenic amines in *Schistosoma mansoni*. *Comparative Biochemistry and Physiology* **39A,** 859–867.

Catravas, G.N., Takenaga, J. & McHale, C.G. (1977) Effect of chronic administration of morphine on monoamine oxidase activity in discrete regions of the brain of rats. *Biochemical Pharmacology* **26,** 211–214.

Chou, T.C.T., Bennett, J.L. & Bueding, E. (1972) Occurrence and concentration of biogenic amines in trematodes. *Journal of Parasitology* **58,** 1098–1102.

Halton, D.W. & Gustafsson, M.K.S. (1996) Functional morphology of the platyhelminth nervous system. *Parasitology* **113,** S47–S72.

Hariri, M. (1974) Occurrence and concentration of biogenic amines in *Mesocestoides corti* (Cestoda). *Journal of Parasitology* **60,** 737–747.

Holmes, S.D. (1984) *In vitro* studies into the mode of action of anthelmintics on the liver fluke *Fasciola hepatica*. PhD thesis, The Queen's University of Belfast, Belfast, UK.

Holmes, S.D. & Fairweather, I. (1984) *Fasciola hepatica*: the effects of neuropharmacological agents upon *in vitro* motility. *Experimental Parasitology* **58,** 194–208.

Johnston, J.P. (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochemical Pharmacology* **17,** 1285–1297.

Knoll, J., Ecsery, Z., Kelemen, K., Nievel, J. & Knoll, B. (1965) Phenylisopropylmethyl propionylamine [E250] a new spectrum psychic energizer. *Archives of International Pharmacodynamics* **155,** 154.

Mansour, T.E. (1962) Effect of serotonin on glycolysis in homogenates from the liver fluke *Fasciola hepatica*. *Journal of Pharmacology and Experimental Therapy* **135,** 94–101.

Mansour, T.E. (1979) Chemotherapy of parasitic worms: new biochemical strategies. *Science* **205,** 462–469.

McGilvery, R.W. & Goldstein, G. (1979) *Biochemistry: a functional approach*. London, W.B. Saunders Co.

Mishra, S.K., Sen, R. & Ghatak, S. (1978) Monoamine oxidase and metabolites of biogenic amines in human *Ascaris*. *Indian Journal of Parasitology* **2,** 153–155.

Mishra, S.K., Sen, R. & Ghatak, S. (1983) Monoamine oxidase in adult *Ascaridia galli*. *Journal of Helminthology* **57,** 313–318.

Mishra, S.K., Agarwal, A., Sen, R. & Ghatak, S. (1985) Characterization of mitochondrial monoamine oxidase of *Ascaridia galli*. *Journal of Helminthology* **59,** 101–107.

Moreno, M.S. & Barrett, J. (1979) Monoamine oxidase in adult *Hymennolepis diminuta* (Cestoda). *Parasitology* **78,** 1–5.

Nimmo-Smith, R.H. & Raison, C.G. (1968) Monoamine oxidase activity of *Schistosoma mansoni*. *Comparative Biochemistry and Physiology* **24,** 403–416.

Podesta, R.B., Siddiqui, A.A. & Ansell, M.T. (1986) Isolation of parasite surface membranes. *In* Hayunga, E.G. & Stek, M. Jr. (*Eds*) *Nuclear and related techniques in parasitology.* Vienna, Austria, International Atomic Energy Agency Publication.

Smart, D. (1988) Catecholamine synthesis in *Ascaridia galli* (Nematoda). *International Journal for Parasitology* **18,** 485–492.

Sokal, R.R. & Rohlf, F.J. (1981) *Biometry: the principles and practice of statistics in biological research*. 2nd edn, W.H. Freeman.

Spector, T. (1978) Refinement of the Coomassie Blue method of protein quantitation. *Analytical Biochemistry* **86,** 142–146.

Terrada, M., Ishii, A.I.H. & Sano, M. (1982) Studies on chemotherapy of parasitic helminths (VII). Effects of various cholinergic agents on the motility of *Angiostrongylus cantonensis*. *Japanese Journal of Pharmacology* **32,** 633–642.

Von Brand, T. (1979) *Biochemistry and physiology of endoparasites*. Amsterdam, Elsevier, North Holland Biomedical Press.

Weissbach, H., Smith, T.E., Daly, J.W., Witkop, B. & Undenfriend, S. (1960) A rapid spectrophotometric assay of monoamine oxidase based on the rate of disappearance of kynuramin. *Journal of Biological Chemistry* **325,** 1160–1163.

(Accepted 4 February 2000)