The digenetic trematodes infest loaches in less turbulent waters whereas nematodes occur in loaches inhabiting hill streams and torrential waters, indicating thus some sort of host specificity related to the permanent habitat of the fish and its dietary requirements. In numerical terms, mortality of loaches caused by parasitism in natural habitats is perhaps only a minute fraction.

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A PRELIMINARY STUDY ON BAIT SHYNESS IN *BANDICOTA BENGALENSIS*, TOWARDS ZINC PHOSPHIDE

UNTIL recently, poison baiting with 2–5% zinc phosphide for the control of rodents was recommended for 2–3 consecutive days[1,2]. But many Indian rodent pests rapidly develop bait shyness on such a poisoning regime[3–5]. Although *B. bengalensis* is one of the most common agricultural and commercial pests in India[6], there is little information on responses of this species towards poisons or on the effects of sub-lethal poisoning on subsequent behaviour. Lesser bandicoot rats were therefore tested for their response to repeated sub-lethal feedings of zinc phosphide and for the possible development of bait shyness.

Ten adult *B. bengalensis*, five of each sex, were kept in individual casges, 60 × 30 × 30 cm, and were provided daily with fresh weighed amounts of polished rice (*Oryza sativa*) and maize (*Zea mays*) in two circular metal dishes fixed at the front of the cages. Drinking water was always available. Total daily food intake was recorded to 0·1 g for eight days. The positions of the foods were alternated daily to prevent the development of position preferences. On each of the next five days, 0·05% zinc phosphide (a sub-lethal dose) and 1% groundnut oil were added to the rice, which was the preferred food. Thereafter rats were fed for six day periods on a pellet diet (Hindustan-Hindlever animal feed); on each seventh day rats were tested for bait shyness by offering them a choice of rice + 1% groudnut oil or maize. Bait shyness was measured by comparing the average preference for rice on the last three days before poisoning (calculated as weight of rice eaten divided by the weight of maize eaten + 0·5, since some rats ate no maize) with preference for rice on the weekly tests after poisoning.

Poisoning the rice with sub-lethal amounts of zinc phosphide resulted in a rapid reversal of preference; preference for rice decreased from a median value of 14·6 on days 5–8 to 0·5 on day 13, the last poisoning day ($p > 0·01$). The decreased preference for rice was immediate, median preference falling to only 4·5 after the first day of poisoning. Such rapid avoidance of poisoned food has previously been noted[1]. On their first test for bait shyness, 9/10 rats still had lower preferences for rice than before poisoning (Table I: $p > 0·02$), but by the third test, the median preference for rice was little different from that of pre-poisoning (Table I). But importantly, some rats still showed continued reduced perference for rice on the third test, an indication of longlasting bait shyness similar to that observed in other Indian rodents[3–5].

TABLE I

Pre- and Post-poisoning preferences for rice of B. bengalensis

Rats	Mean pre-poison preference	Post-poison Preference Tests 1	2	3
1	1·5	1·1	5·4	18·0
2	26·6	52·8	44·0	28·1
3	11·3	0·8	1·0	5·4
4	1·1	0·8	1·5	1·3
5	13·1	3·6	4·8	2·8
6	17·4	12·2	4·6	18·1
7	9·0	4·6	10·5	9·4
8	16·0	0·8	1·2	1·4
9	21·8	2·1	2·0	11·4
10	18·0	4·4	19·6	21·3
Medias	14·6	2·9	4·7	10·4

(Values are g consumed/100 g body weight).

There was much individual variation in response to repeated sub-lethal feedings on zinc phosphide. Such variation in behaviour is probably the main source of problems in rodent control using acute rodenticides[3,4] both because some animals never ingest lethal amounts of poison and because a proportion of the

population rapidly develops bait shyness. As previously suggested, therefore, poison baiting with zinc phosphide should be carried out for only one day after pre-baiting; if any residual population is to be controlled immediately, a different bait and poison should be used or burrow fumigation should be attempted[5].

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COLORIMETRIC METHOD FOR THE DETERMINATION OF HUMAN SERUM CHOLINESTERASE AND ITS ACTIVITY TOWARDS METHYL PARAOXON

Introduction

A COLORIMETRIC method has been developed for assaying cholinesterase activity in the human serum using P-nitrobenzene diazonium fluoborate as the chromogenic reagent. The method was found to be sensitive and simple. The cholinesterase enzyme sensitivity towards methyl paraoxon was tested. The per cent cholinesterase inhibition at different concentrations of methyl paraoxon was determined. The colorimetric method might be employed in clinical assay of cholinesterase activity during organophosphorus pesticide poisoning.

Colorimetric methods for the determination of cholinesterase activities in human serum have been reported earlier using tetraazotized diorthoanisidine (naphthanil Diazo Blue B), as chromogenic reagent[1]. The chromogenic reagent 'Fast B Blue' is employed for the detection of organophosphorus pesticides on silica gel in thin layer chromatography by cholinesterase inhibition[2-4]. The authors reported a rapid and simple quantitative method for the determina ion of organophosphatic pesticides on thin layer chromatography by cholinesterase inhibition using Fast B Blue as the chromogenic reagent[4]. Subsequently, the authors reported a simple, sensitive and rapid method for the detection and quantification of paraoxon on thin layer chromatography by cholinesterase inhibition using *p*-nitrobenzene diazonium fluoborate as the chromogenic reagent[5]. The colorimetric method employed is suitable only for thin layer chromatogaphic-enzymatic method[5]. Whereas in the present, colorimetric method is described employing *p*'-nitrobenzene diazonium fluoborate for assaying in a reaction mixture with human serum as the enzyme source. Investigations also show the relationship between cholinesterase inhibition and methyl paraoxon concentration *in vitro* in human serum. The method may find clinical application in cholinesterase assay for the diagnosis of pesricide poisoning due to contamination.

Material and methods

The oxygen analogue for methyl parathion (O,O,-Dimethyl O-4-nitrophenyl thiophosphate) (99% pure) was obtained from CIBA-Geigy Ltd., Basel was prepared as reported earlier[5]. Methyl paraoxon was chosen as it is more soluble in water than ethyl parathion. The solubility of methyl parathion is 55 mg/1 and its oxygen analogue is highly soluble in water as compared with its parent compound[6].

Fresh citrated human blood was obtained from the blood bank, and serum was isolated. The serum was preserved at 0°C.

1·0 ml of reaction mixture, consisted of 0·1 ml of serum, 6·0 μ moles of 2-naphthyl acetate (Koch-Light Laboratories, England) in 0·01 ml of acetone, and 1 to 8 μ gm of methyl paraoxon in distilled water. The controls contained distilled water in place of methyl paraoxon and 0·01 ml of acetone. The reaction mixtures were pre-incubated for 8 minutes at 28°C with methyl paraoxon. After this preincubation, 2-naphthyl acetate was added and the mixture incubated for 5 more minutes. The enzymic reaction was stopped by the addition of 4·0 ml of glacial acetic acid, followed by 0·2 ml of 0·4% of *p*-nitrobenzene diazonium fluoborate in acetone and the samples were allowed to stand for 30 minutes at room temperature. The orange brown colour complex formed due to diazocoupling reaction between 2-naphthol and dia-