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-

zonium salt, was measured at 500 nm using a Beckman DK 2A Ratio Recording Spectrophotometer. The optical density values of the experimental (with methyl paraoxon) and control (without methyl paraoxon) samples were compared and the per cent cholinesterase inhibition was calculated.

Results and Discussion

For assaying the cholinesterase activity of the human serum, the amount of organic solvent in the reaction mixture was kept to the minimum. For this reason the methyl paraoxon was prepared in distilled water, as its solubility in water is more than that of parathion, whereas 2-naphthyl acetate was added in 0·01 ml of acetone as this volume of acetone did not cause any turbidity in the reaction mixture. Addition of 0·04 ml of acetone caused slight turbidity. The colour formed due to coupling reaction described early is stable indefinitely.

The initial velocity of the enzyme reaction was studied with respect to time, substrate and these factors were appropriately chosen for assaying the cholinesterase activity. The results show that per cent cholinesterase inhibition increased with the increase in the methyl paraoxon concentration (Table 1).

TABLE I

Effect of methyl paraoxon on human serum cholinesterase activity

No. of observations in each case—12.

Concentration of methyl paraoxon μgm/ml	Per cent ChE inhibition
0·5	+10± 3
1	−18± 5
2	−43± 5
3	−54± 3
4	−63±11
5	−67±11
6	−75±15
7	−74± 4

Enzyme activity 100% = 29·2 μm. 2-naphthyl-acetate/ml of serum/hr.

+ % Activation over control; − % Inhibition over control; ± Standard Deviation of mean.

However below 1 μgm. ($4·2 \times 10^{-6}$ mole) of methyl paraoxon, the cholinesterase activity showed a slight increase (Table 1)) The enzyme activation at a very low concentration of methyl paraoxon, *i.e.*, 0·5 μgm. ($2·1 \times 10^{-6}$ mole) can be corroborated with the results reported earlier[7] wherein similar cholinesterase activation at concentrations of 5×10^{-5} to 5×10^{-7} mole of methyl paraoxon in the liver of monkey was observed. The cause of the activation at such low concentrations has not been clearly explained[7]. The cholinesterase inhibition showed variation upto 15% from the standard mean values in a few observations. This might be attributed to the variation in the cholinesterase levels of the individuals themselves. For example, repeated tests over short term and long term periods ranging from one week to five years on humans have shown variation in the cholinesterase activity levels from 7 to 11·3% as observed by several investigators[8–11].

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A SIMPLE MEDIUM TO DETECT L-ASPARAGINASE POSITIVE BACTERIA

L-ASPARAGINASE (L-asparagine amidohydrolase E.C. 3.5.1.1.) is known to possess anti-leukaemic properties in animals[1] and in human beings[2]. In order to meet the requirements for clinical use, the enzyme has to be produced in larger quantities. This prompted many researchers to screen large numbers of bacteria for L-asparaginase activity[3–7].