

IN VIVO INHIBITION OF RAT BRAIN MONOAMINE OXIDASE BY SELECTED PESTICIDES

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ABSTRACT

In vivo effects of selected pesticides on rat brain monoamine oxidase (MAO) activity have been studied after 1 hr of administration of a single oral dose at the level of 50% of LD₅₀ as well as after 15 days and 60 days of administering daily one dose of each pesticide at the level of 5% of LD₅₀. However irrespective of acute or chronic administration all pesticides inhibited brain MAO activity, though the degree of inhibition was less pronounced after the long-term (60 days) administration, suggesting development of some detoxifying mechanism. Of the pesticides administered organophosphates were found to be more potent inhibitors.

INTRODUCTION

MONOAMINE oxidase (EC 1.4.3.4; MAO) is a flavoprotein enzyme, located predominantly on the outer mitochondrial membrane. It catalyses the breakdown, by oxidative deamination, of a variety of biogenic amines and thus regulates their levels in the tissues.

MAO inhibition by pesticides has been reported¹⁻³ to be directly proportional to the length of the alkyl chain of the pesticides. However, in earlier studies, the effects of pesticides upon MAO were examined after a single fixed period of treatment which varied depending upon the workers.

In the present paper, an attempt is made to determine whether or not there are any major differences in the effects of short and long-term administration of carbamate as well as organophosphate groups of pesticides upon rat brain MAO activity.

MATERIALS AND METHODS

Healthy male albino rats weighing 150–250 g each were taken for acute studies and weanling albino rats of almost the same age and size, weighing 50–70 g, were selected for the chronic study. Rats were starved for 12 hr prior to experiment. The animals were given rat feed ("Gold mohur" of Hindustan Lever Limited, Ghaziabad) and water *ad libitum* throughout the period of study.

The acute administration effects of the pesticides was studied by dividing the rats into five groups of five animals each. Animals of one group were orally

given the minimum amount of groundnut oil and served as control. Rats of the remaining four groups were orally administered separate pesticides (dissolved in the same volume of groundnut oil) at the level of 50% of LD₅₀ doses⁴⁻⁶. Each animal of the five groups was killed after 1 hr of pesticide treatment.

For chronic study, rats were divided into ten groups of five animals each. Each pesticide was dissolved/suspended, at the level of 5% of the LD₅₀ dose, in a minimum volume of groundnut oil and was administered daily to two groups of rats with the help of a catheter. The remaining two groups of animals were orally given the same volume of groundnut oil which served as control. Of the two groups of animals for each pesticide the rats of one group were killed upon completion of 15th day along with one of the two control groups of rats. The animals of the second group for each pesticide were continued to be treated with pesticides up to the 60th day and then killed along with the remaining control group.

Rats were killed by decapitation. Brain was removed immediately and washed in chilled normal saline, blotted, dried and weighed. Each brain was homogenized in chilled isotonic medium (0.25 M sucrose, 0.01 M tris and 0.001 M EDTA) to form 10% homogenate and centrifuged at 650 g for 10 min to remove cellular debris. MAO activity was estimated in the clear supernatant by the method of Ono *et al*⁷ using *p*-benzylamino-azo- β -naphthol (0.5 mM) as a substrate and *p*-benzaldehyde-azo- β -naphthol (15 μ g/ml) as standard. The protein content of the supernatant was estimated according to Lowry *et al*⁸.

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Statistical significance of differences between mean MAO activities of control and the corresponding pesticide-treated groups of rats was tested by students *t* test⁹.

RESULTS

In the adult albino rats of the control group, the brain MAO activity was of the order of 22.42 ± 1.44 units per mg protein. Acute administration of all the four pesticides resulted in considerable inhibition of MAO activity. The observed percentage inhibitions were as follows: 33.6 (carbaryl), 46.0 (bavistin), 44.0 (elsan) and 52.0 (phosalone), which were all statistically highly significant ($P < 0.001$) (table 1; figure 1).

The control group weanling albino rats used along with the chronic administration groups exhibited MAO activity of the order of 23.99 ± 2.15 units/mg protein on the 15th day. Chronic administration of each pesticide for 15 days also brought about inhibition of MAO activity but to a lesser degree in comparison to acute dose-administered groups. Carbaryl and bavistin, brought about less significant ($P < 0.01$) inhibition, whereas, elsan and phosalone resulted in highly significant ($P < 0.001$) inhibition of MAO activity (table 1; figure 2).

The second control group of animals exhibited MAO activity of the order of 26.35 ± 2.06 units per mg of protein on 60th day. Continued administration of these pesticides at the same dose (5% of LD_{50}) for 60 days revealed lesser inhibition of MAO activity in comparison to 15 days exposed groups. The observed degree of inhibition was of the order of 18.3% (carbaryl), 17.5% (bavistin), 20.1% (elsan) and 29.3% (phosalone) (table 1; figure 2).

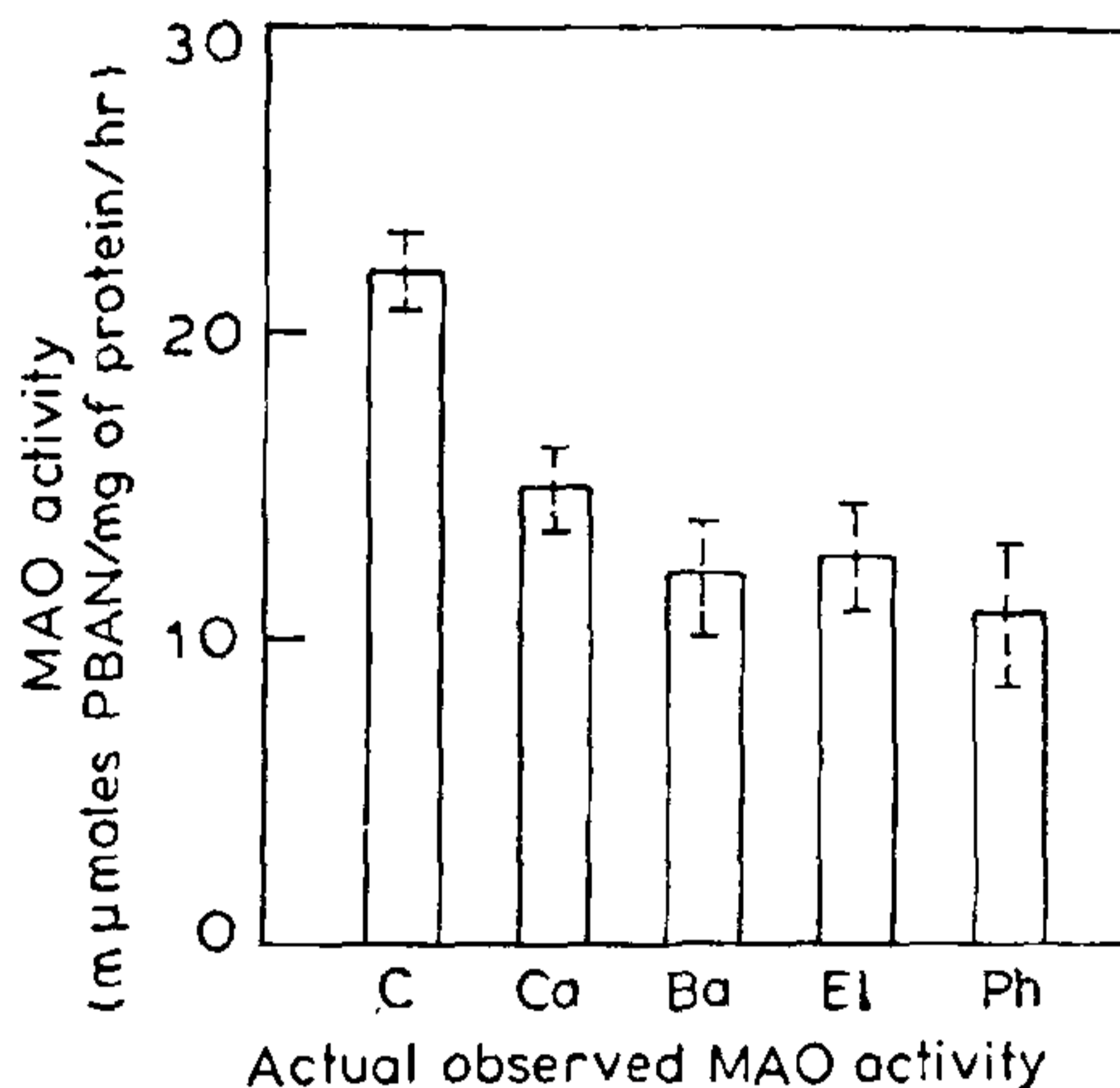
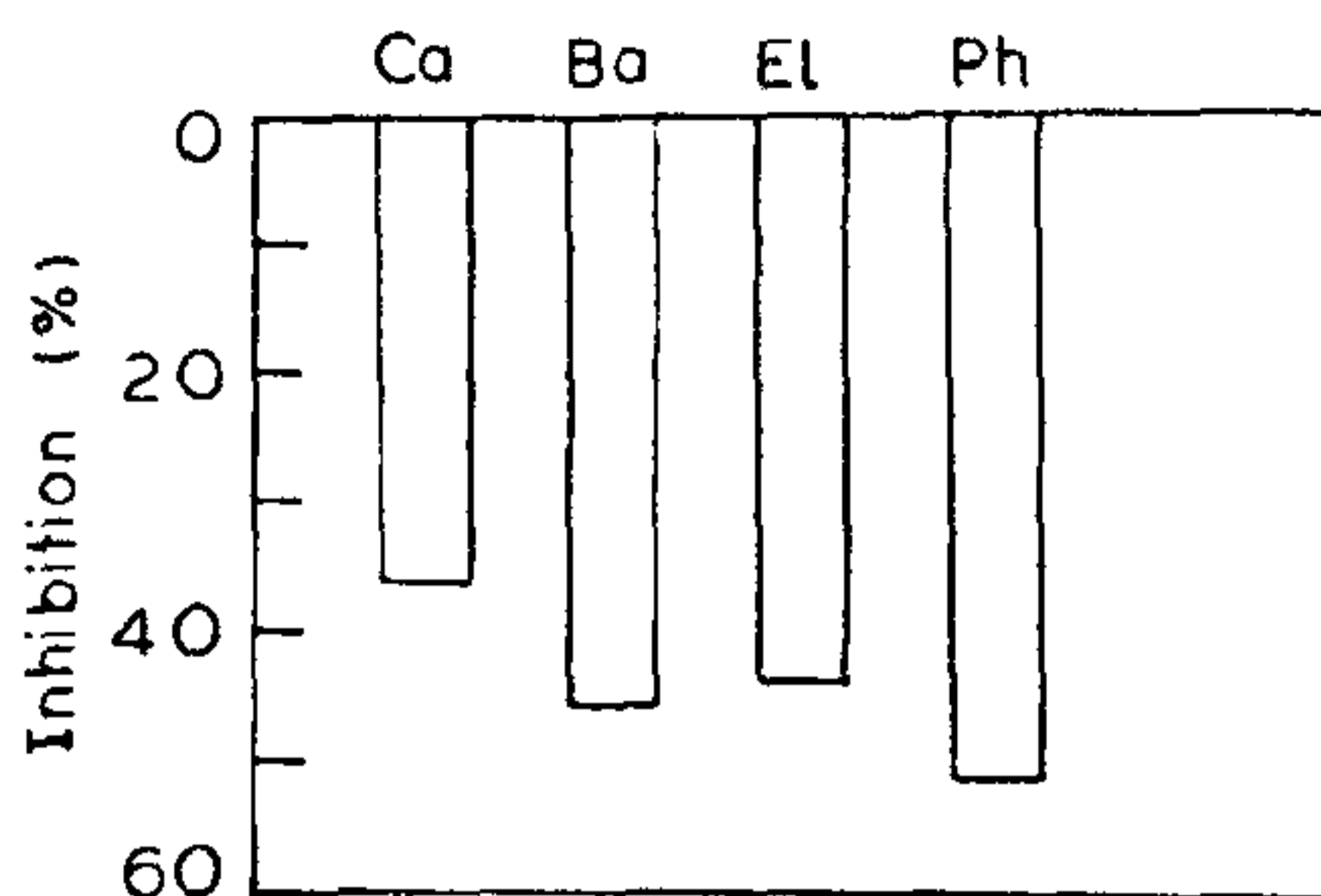


Figure 1. Effect of acute administration of different pesticides (50% LD_{50}) on brain monoamine oxidase activity in the albino rats.

Table 1 Effect of acute and chronic administration of pesticides on brain monoamine oxidase (MAO) activity in the rats

Treatment period	Monoamine oxidase activity* (Mean \pm SD)				
	Control group	Carbaryl fed group	Bavistin fed group	Elsan fed group	Phosalone fed group
1 hour	22.42 ± 1.44	14.88 ± 1.46^c (33.6)	12.16 ± 1.91^c (46.0)	12.58 ± 1.64^c (44.0)	10.85 ± 2.16^c (52.0)
15 days	23.99 ± 2.15	18.68 ± 1.98^b (22.2)	17.99 ± 2.04^b (25.1)	17.61 ± 1.49^c (26.6)	16.04 ± 1.38^c (33.2)
60 days	26.35 ± 2.06	21.53 ± 3.74^a (18.3)	21.75 ± 3.73^a (17.5)	21.05 ± 3.29^a (20.1)	18.63 ± 2.80^c (29.3)

Number of rats in each group was five; * Specific activity of the enzyme was defined as the production of number of μmol of *p*-benzaldehyde-azo- β -naphthol (PBAN) per hour per mg of protein at 37°C ; per cent inhibition in enzyme activities are represented in figures in parentheses; $a = P < 0.05$; $b = P < 0.01$; $c = P < 0.001$; vs corresponding group.

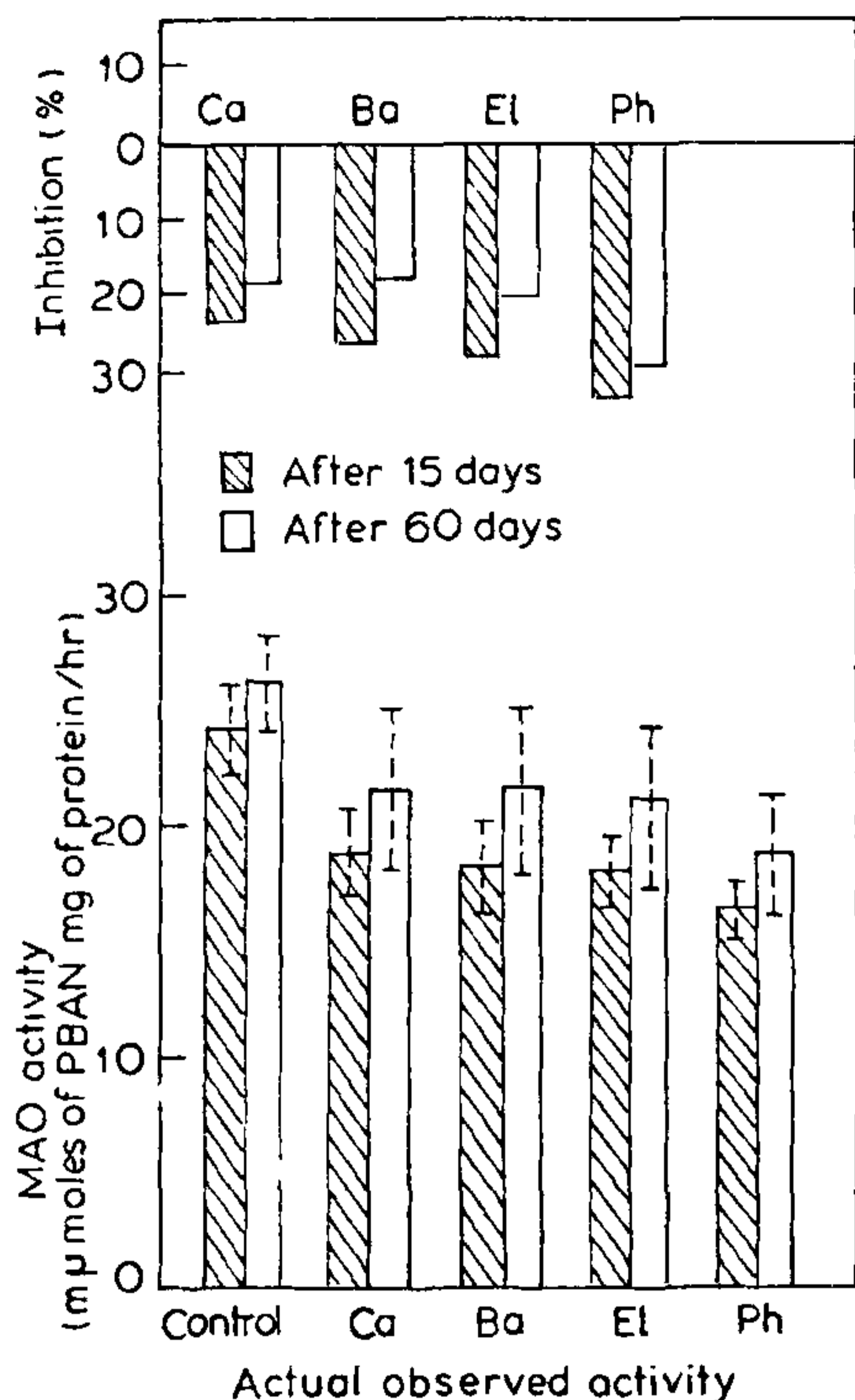


Figure 2. Effect of daily administration of pesticides for 15 days and 60 days at the level of 5% of LD_{50} dose on monoamine oxidase activity in the albino rats.

DISCUSSION

Surprisingly, the pesticides used in the present study, both of carbamate as well as organophosphate groups, have been found to significantly inhibit MAO activity in the rat brain tissue *in vivo*, though organophosphate pesticides appeared to be more potent MAO inhibitors.

The majority of the active organophosphate compounds possessing a substituted coumarinyl moiety, a diethyl moiety, a dimethyl moiety and a 'P = S' or 'P = O' moiety has been reported¹ to be MAO inhibitory in nature. The organophosphates, viz phosalone and elsan used in the present study, also contain a diethyl moiety or a dimethyl moiety, and 'P = S moiety' in their molecular structure. However, elsan inhibits MAO activity to a slightly lesser extent as compared to phosalone. This could

be due to the length of the alkyl chains present in these inhibitors. Phosalone contains diethyl moiety whereas elsan contains dimethyl group. MAO inhibition has been reported² to be directly proportional to the length of the alkyl chain. This interesting structure-activity relationship among the pesticides has also been supported by a documented³ evidence indicating that dimethyl chlordimeform is a more potent pesticide as compared to chlordimeform. It has been reported¹ that N-methyl carbamyl moiety present in carbaryl may be responsible for MAO inhibitory activity. These inhibitors are bound to cause a rise in the levels of biogenic amines *in vivo* and cause neurological disorders associated with the raised levels of these amines.

The probable reason for the enhanced anti-MAO properties of organophosphates over carbamates may be the presence of phosphoryl moiety in the former which might facilitate binding to the active site of MAO. However, conclusions relative to the active site interactions of organophosphates and carbamates examined in this study appeared unwarranted as a consequence of the MAO heterogeneity of the enzyme preparation.

However, the decline in the degree of MAO inhibition in the weanling rats upon prolongation of the duration of feeding of pesticides, at the same dose, from 15 to 60 days is probably suggestive of development of some alternate mechanism in the body trying to nullify the effect of the pesticides. This mechanism could be the production of some inducible enzyme(s) in the liver for which pesticide itself might be working as inducer as well as substrate. This induced enzyme(s) could be detoxifying the pesticide into some such metabolite(s) which is (are) less potent MAO inhibitors.

Whether MAO inhibition is responsible for any of the lethal or sublethal effects observed in animals following exposure to pesticides is uncertain at present. However, carbaryl¹⁰ and chlordimeform¹¹ were shown to produce changes in biogenic amine metabolism when administered to rats. Thus interference with biogenic amine regulatory mechanisms by pesticides may be of some consequence.

ACKNOWLEDGEMENT

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NEWS

METHOD DEVELOPED TO INFLUENCE GENES

Academician Sergei Gershenzon and his colleagues at the Academy of Sciences of the Ukraine, have discovered a regularity important for the selection of agricultural animals and plants.

It is known that physical or chemical impacts can evoke in a live organism a change in genetic information (mutation). This leads to accidental, unpredictable changes in the cell's genetic apparatus which, in most cases, are harmful to the organism. Sergei Gershenzon and his colleagues have developed a method of selective influence on separate genes to obtain the present change in the organism's

properties. It has also been established that as different from all known mutagens, DNA, separated from biological objects (mammals, birds, fish, insects, plants and viruses), and influencing other organisms, evokes in them a strictly definite set of mutations. Research has shown that not only natural DNA but also their synthetic analogues have the same property. (*Soviet Features*, Science and Technology, Information Department, USSR Embassy in India, P.B. No. 241, 25, Barakhamba Road, New Delhi 110 001.)
