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Effect of diacetylmonoxime and atropine on malathion-induced changes in blood glucose level and glycogen content of certain brain structures of rats

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The organophosphorous compounds inhibit cholinesterase and increase the level of acetylcholine, which stimulates the central nervous system and causes hyperactivity or tremors and induces convulsions in excessive amounts [1–4]. Hyperglycaemia is another manifestation of toxicity induced by certain organophosphorous compounds [5, 6]. The oximes have been reported to control the toxic effects induced by organophosphate compounds by reactivating the phosphorylated (inhibited) cholinesterase [7–9]. The effect of oximes on the hyperglycaemia induced by organophosphorous compounds has not been determined. The aim of the present study was to determine the effect of diacetylmonoxime (DAM) and atropine on the level of blood glucose in malathion-treated rats.

Certain tremorigenic agents, for example oxotremorine [10], have been reported to reduce the level of cerebral glycogen. Since tremors appear early during the course of poisoning induced by organophosphorous compounds, we also determined the effect of malathion and other drugs on the glycogen content of various brain structures including the corpus striatum (which is mainly involved in the production of tremors [11, 12]).

Adult male albino rats, 175 ± 15 g, were used. The animals were fasted for 18 hr before use since preliminary experiments indicated that more uniform results were obtained in this manner. The animals were injected with malathion, 500 mg/kg, i.p. Some of the malathion-treated animals were injected with DAM (100 mg/kg, i.p.) or atropine (25 mg/kg, i.p.) immediately or 30 min after the administration of malathion. Controls received normal saline. Malathion was also administered to rats that had received reserpine (1 mg/kg, i.p.) daily for 3 days. The animals were decapitated 1 hr after treatment with malathion. The skull was opened immediately, and various brain regions were quickly separated [13]. The blood glucose level was determined by the method of Nelson [14]. The glycogen was extracted according to the method of Lebaron [15] and estimated colorimetrically as described by Montgomery [16].

The data were analysed statistically using Student's *t*-test, and significant differences between the means were determined.

The values of blood glucose and of glycogen in various brain structures of malathion-treated rats are given in Table 1. The level of blood glucose was raised, and glycogen in various brain structures was reduced after treatment with malathion (Table 1). The changes in the levels of blood glucose and cerebral glycogen were not modified by DAM or atropine given 30 min after the administration of malathion; treatment with these drugs immediatley after malathion prevented the increase in blood glucose and the depletion of glycogen in various brain structures (Table 1). Pretreatment with reserpine did not modify the induced changes in blood glucose and cerebral glycogen in malathion-treated animals.

It was reported previously that organophosphorous compounds induced hyperglycaemia through the release of catecholamines which have a known hyperglycaemic effect [17]. Our results indicate that treatment with reserpine, a known depletor of catecholamines [18], failed to prevent the increase in blood glucose level in malathion-treated rats (Table 1). Other workers have also reported that α-adrenergic blockade prevented the catecholamine-induced hyperglycaemia [19] but did not abolish the organophosphate-induced hyperglycaemia [6]. Thus, other mechanisms may be involved in the production of hyperglycaemia in malathion-treated animals.

It was reported previously that DAM readily crossed the blood–brain barrier and was more effective than other oximes in reactivating the cholinesterase in the brain [1, 20]. Accordingly, DAM was used in the present study to obtain quick reactivation of cholinesterase activity in the brain. The results presented indicate that DAM given 30 min after the administration of malathion did not restore the induced hyperglycaemia to normal; however, DAM or atropine given immediately after malathion prevented the hyperglycaemia and depletion in the level of glycogen in various brain structures of malathion-treated animals (Table 1). The drugs may have been less effective when administered 30 min after malathion treatment since, at that time, the rise in blood glucose, possibly mediated through acetylcholine, would most likely have been fully established.

Certain organophosphorous compounds (e.g. Soman) have been reported to increase the level of cyclic AMP in the brain [21]. Cyclic AMP is believed to regulate the storage of glycogen which is hydrolysed or reduced with the rise in blood glucose level [22]. According to certain workers, the level of cerebral glycogen is increased after anesthesia and the administration of certain barbiturates and sedatives [23, 24]. Thus, the level of cerebral glycogen seems to be influenced by the state of activity of the brain. The reduction in the level of glycogen in various brain structures (Table 1) may have been related to the stimulatory effects associated with an increase in the concentration of acetylcholine [1–4] or of cyclic AMP [21], induced by organophosphorous compounds. It is also possible that

Table 1. Effect of DAM (100 mg/kg, i.p.), atropine (25 mg/kg, i.p.) and reserpine (1.0 mg/kg, i.p. daily for 3 days) on the blood glucose level and the glycogen content of certain brain structures of malathion-treated rats*

Treatment	Blood glucose (mg/100 ml)	Glycogen (mg/100 g)			
		Cerebral cortex	Corpus striatum	Cerebellum	Medulla
(1) Controls	95.1 ± 2.9	43.1 ± 1.3	73.5 ± 4.2	81.2 ± 3.7	96.3 ± 4.4
(2) Malathion	215.7 ± 10.9†	35.2 ± 1.6†	52.8 ± 2.7†	66.1 ± 3.9†	73.8 ± 2.9†
(3) Malathion + DAM (immediately)	108.4 ± 6.7‡	44.9 ± 2.1§	75.1 ± 3.5‡	79.7 ± 2.8‡	94.5 ± 3.4‡
(4) Malathion + DAM (after 30 min)	202.12 ± 11.3†	36.9 ± 1.7‖	54.5 ± 2.9†	67.7 ± 4.1†	76.1 ± 3.2†
(5) Malathion + atropine (immediately)	100.9 ± 5.9‡	42.5 ± 1.9§	70.9 ± 3.8‡	80.5 ± 3.4‡	97.3 ± 4.1‡
(6) Malathion + atropine (after 30 min)	190.0 ± 8.5†	37.1 ± 1.8‖	56.1 ± 2.5†	68.9 ± 2.8†	77.2 ± 2.6†
(7) Reserpine + malathion	207.6 ± 12.2†	34.6 ± 1.5‖	50.7 ± 3.1†	65.3 ± 2.6†	75.8 ± 3.6†

* Values are the means ± S.E. of six animals in each group. The animals in each group were killed 1 hr after treatment with malathion (500 mg/kg, i.p.).
† Significantly different from the control values, group 1 ($P < 0.01$).
‡ Significantly different from the values in malathion-treated animals, group 2 ($P < 0.01$).
§ Significantly different from the values in malathion-treated animals, group 2 ($P < 0.05$).
‖ Significantly different from the control values, group 1 ($P < 0.05$).

enhanced cholinergic activity by malathion may induce the release of certain humoral factors or somatostatin which might be involved in the mobilization of glycogen leading to hyperglycaemia [25–27]. The possible involvement of a cholinergic mechanism is suggested by the finding that atropine, a known blocker of cholinergic activity, abolished the hyperglycaemia and reduction in the level of glycogen in various brain structures of malathion-treated rats (Table 1).

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Filter trapping of $^{14}CO_2$: a simple and quantitative method for studying cell metabolism in hepatocyte monolayers

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Quantitative analysis of $^{14}CO_2$, appearing in the exhaled air after administration of specifically ^{14}C-labelled aminopyrine has been extensively used as a noninvasive method to study oxidative xenobiotic metabolism *in vivo* both in animals [1–4] and in man [5–8]. Although an *in vitro* application of this approach was shown to be feasible in hepatocyte suspensions [9, 10], its widespread use has been hampered owing to the lack of a simple, yet quantitative CO_2 collection system. Since, compared to hepatocyte suspensions, monolayer cultures offer several advantages (e.g., recovery from isolation procedure, cell to cell contact, prolonged viability), we have developed and tested a CO_2 collection system in chick embryo hepatocyte monolayers. This paper focuses on the use of the system for metabolic studies with ^{14}C-labelled aminopyrine; however, it is basically applicable to the study of a wider variety of compounds, which contain *N*-methyl moieties and yield C_1 fragments during biotransformation.

Materials and methods

Preparation of hepatocyte monolayers. The livers of 16 days old chick embryos (crossbred shaver strain) were prepared according to the method of Granick *et al.* [11] as modified by Meyer *et al.* [12]. The collagenase used was purchased from Millipore Corp., (Freehold, NJ) the Hanks BSS without Ca^{2+} and Mg^{2+} from Gibco-Biocult (Paisley, U.K.). The hepatocytes were plated at a density of 2.4×10^5 cells/cm^2 in Falcon T25 culture flasks (Becton-Dickinson,