Effects on Esterases and Comparison of I_{50} and LD_{50} Values of Malathion in Suckling Rats

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Previous studies showed that young rats were more susceptible to malathion than the older ones (Lu et al, 1965; Brodeur and DuBois, 1963 and 1967; Mendoza, 1976). Based on LD_{50} values, 17-day old rats were 9 times more resistant to malathion intoxication than 1-day old pups (Mendoza, 1976). The inhibition of esterases were also shown to be inversely related to age. Esterases in the liver, brain and kidney of 1-day old pups were much more susceptible to inhibition by malathion than those of 18-day old pups. For example, 1-day old pups require only 500 mg/kg malathion while 18-day old pups required 4000 mg/kg to effect 70% inhibition of liver esterase activity towards indophenyl acetate (IPA). Similarly, 1-day old pups required only 500 mg/kg malathion while 18-day old pups require about 8000 mg/kg to produce about 85% inhibition of brain esterase activity towards acetylthiocholine (ASChI).

The present report deals with further studies of the effects of malathion on esterase activity in 6 and 12 day old pups. In addition, malathion I_{50} based on brain acetylcholinesterase inhibition was determined to establish its relationship with malathion LD_{50} obtained for various ages of rats[a].

METHODS

Virgin female Wistar rats (Biobreeding Laboratories, Ottawa, Canada) were mated and fed standard rat cubes ad libitum. As previously done (Mendoza, 1976), the number of pups per litter was adjusted to 8 or 10 by removal or adoption of the excess pups. The pups in a litter were not segregated according to sex. Previous studies on esterases (Mendoza, 1976) and porphyrin induction (Mendoza et al., 1975) showed no sex difference at the suckling stage in the rat.

[a] I_{50} is the concentration of the inhibitor required to give 50% inhibition of enzyme activity at specified conditions. LD_{50} = median lethal dose; a dose that is lethal for 50% of the test subjects.

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One group of litters was dosed by intragastric intubation with malathion on the 6th day after birth and another group was dosed on the 12th day (see Mendoza, 1976). Malathion (99.3% pure), (American Cyanamid Co., Wayne, New Jersey) dissolved in corn oil (Mazola) was used for dosing. The control pups were given corn oil only or not treated at all. The control and treated pups received the same level of corn oil, 17 mg/kg body weight. The oil dose did not cause any observable difference between those receiving and not receiving oil. Malathion dosage ranged from 250 to 2000 mg/kg or 500 to 4000 mg/kg for the 6- or 12-day old pups, respectively. These dosage ranges were used to meet the requirement specified in Weil's method (1952). Two animals per dose were used and the tests were replicated 4 times per age group.

After dosing, the pups were returned to their respective dams and were observed hourly for the first 5 hr. The dead pups were refrigerated at about 4°C pending dissection and the survivors were decapitated after 24 hr. The liver, brain and kidney from all the pups were excised, rinsed with ice-cold 0.9% NaCl solution, blotted, weighed and stored in a freezer pending enzyme analysis. Previous studies showed that extended storage in a frozen condition has no effect on esterase activities (Mendoza et al., 1971).

The enzyme activity towards IPA, ASChI, and butyrylthiocholine (BSChI) was determined according to the previously published method (Mendoza, 1976) using a Technicon Autoanalyzer System (Montreal). The enzymatic method used was basically that of Ellman et al. (1961) and Gary and Routh (1965) for thiocholine and that of Mendoza et al. (1971 and 1972) for IPA. The enzyme activity was expressed in μmol/min/g wet tissues.

I_{50} values were estimated by plotting the malathion dose in a logarithmic scale and percent enzyme inhibition in a probit scale (c.f. Mendoza and Shields, 1973).

RESULTS AND DISCUSSION

Table I shows the enzyme activity in the liver, brain and kidney of 7-day old rats with or without malathion treatment. At a 250 or 500 mg/kg malathion dose, approximately 65% inhibition of liver esterase activity towards IPA and 13 to 36% inhibition of liver cholinesterase activity were observed. At 1000 to 2000 mg/kg, inhibition of the liver esterases studied did not exceed 80%. In the brain, however, 90% inhibition of ASChI hydrolysis was observed in rats treated with 1000 to 2000 mg/kg malathion. A very low degree of inhibition of esterase hydrolyzing IPA and only about 50% maximum inhibition were observed in the kidney treated with the 1000 to 2000 mg/kg dosage.

TABLE I

Enzyme activity ± S.E. (μmol of substrate hydrolyzed/min/g wet tissue) in organs of 7-day old rats dosed with malathion by gastric intubation.[a]

Substrate[b]	mg malathion/kg of body weight 0	250	500	1000	2000
			LIVER		
ASChI	539 ± 24	470 ± 45	349 ± 47	122 ± 31	121 ± 21
BSChI	441 ± 17	368 ± 35	281 ± 50	155 ± 22	172 ± 16
IPA	5498 ± 513	1910 ± 319	1836 ± 228	1482 ± 118	1246 ± 184
			BRAIN		
ASChI	1230 ± 54	1038 ± 31	885 ± 170	107 ± 56	110 ± 20
BSChI	122 ± 15	109 ± 13	102 ± 7	59 ± 27	37 ± 6
IPA	1457 ± 94	1257 ± 94	1152 ± 194	1124 ± 200	1112 ± 141
			KIDNEY		
ASChI	631 ± 69	632 ± 15	482 ± 56	283 ± 69	315 ± 44
BSChI	499 ± 59	470 ± 18	383 ± 26	252 ± 57	298 ± 38
IPA	7455 ± 602	8182 ± 663	7240 ± 353	5212 ± 689	6530 ± 289

[a] S.E. = standard error of the mean.

[b] ASChI = acetylthiocholine iodide; BSChI = butyrylthiocholine iodide; IPA = indophenyl acetate.

TABLE II

Enzyme activity ± S.E. (μmol of substrate hydrolyzed/min/g wet tissues) in organs of 13-day old rats dosed with malathion by gastric intubation.[a]

Substrate[b]	mg malathion/kg of body weight 0	500	1000	2000	4000
			LIVER		
ASChI	667 ± 277	292 ± 42	209 ± 77	113 ± 23	89 ± 18
BSChI	424 ± 69	260 ± 42	163 ± 68	137 ± 17	128 ± 13
IPA	4102 ± 256	2237 ± 30	1894 ± 581	1993 ± 503	1553 ± 343
			BRAIN		
ASChI	1358 ± 130	1103 ± 62	711 ± 166	472 ± 79	229 ± 72
BSChI	110 ± 4	126 ± 9	46 ± 24	46 ± 8	39 ± 15
IPA	1130 ± 30	1153 ± 62	830 ± 142	869 ± 79	865 ± 45
			KIDNEY		
ASChI	507 ± 16	292 ± 32	319 ± 89	240 ± 46	226 ± 32
BSChI	358 ± 24	327 ± 21	347 ± 76	264 ± 50	255 ± 32
IPA	8128 ± 142	8325 ± 638	7876 ± 956	6620 ± 499	6642 ± 482

[a] S.E. = standard error of the mean.

[b] ASChI = acetylthiocholine iodide; BSChI = butrylthiocholine iodide; IPA = indophenyl acetate.

TABLE III

I_{50} and LD_{50} values for pups[a] treated by gastric intubation with malathion (99.3% pure).

Age (day)	I_{50}[b]	LD_{50}[c]
1-2	220(25°C)	209 (177-250)
6-7	640(25°C)	707 (0)
6-7	680(37°C)	
12-13	900(25°C)	1085 (841-1415)
12-13	1100(37°C)	
17-18	1800(25°C)	1806 (1415-2003)
17-18 (Control)[d]	1650(25°C)	1415 (0)[e]
17-18 (HCB treated)[d]	3400(25°C)	3317 (2594-3394)[e]

[a] I_{50} and LD_{50} in mg of malathion per kg body weight, 24 hr after the treatment.
I_{50} was determined based on brain acetylcholinesterase activity.

[b] Figure in the parenthesis indicates the temperature during the determination of enzyme activity.

[c] Cited in Mendoza (1976) and figures in the parenthesis indicate the LD_{50} ranges.

[d] Tested simultaneously to evaluate the effects of hexachlorobenzene (HCB) on malathion LD_{50}.

[e] Cited in Mendoza and Shields (1976).

In Table II, the data show that the liver esterase hydrolyzing IPA of the 13-day old pups was slightly less inhibited than that of the 7-day old rats. The rate of inhibition of the liver cholinesterases was slightly higher in the 13-day old than the 7-day pups. Although a higher malathion dosage range of 500-4000 mg/kg was used for the 13-day pups, the pattern of the enzyme inhibition in the brain was similar to that of the 7-day old pups at a dosage range of 250-2000 mg/kg. It should be noted that at 500 mg/kg malathion the 13-day old pups showed no inhibition of brain esterase activity towards BSChI or IPA and very slight inhibition of acetylcholinesterase activity towards ASChI. The inhibition rate of the esterases in the kidney was lower than that observed in the liver or brain.

The results presented in Tables I and II show that esterases of 13-day old pups are less inhibited by malathion than those of 7-day old pups although the

patterns of esterase inhibition rates are similar for both. Furthermore, the dosage range required to obtain I_{50} values in terms of brain acetylcholinesterase inhibition is higher for the 13-day old than for 7-old pups. The results further confirm that 13-day old pups were more resistant to malathion intoxication than 7-day old pups. This observation agreed with the previous report on the 1- and 17-day old pups treated with malathion (Mendoza, 1976).

Table III shows the similarity of I_{50} and LD_{50} values for malathion in the pups of different ages. The data also illustrate the similarity of I_{50} and LD_{50} values of malathion in pups treated with HCB. Although the I_{50} value of 900 mg/kg for 12 to 13-day old pups was lower than the mean LD_{50} value, it is still within the range of LD_{50} values. The data indicated that I_{50} values obtained with brain AChI after intoxication with malathion are useful criteria to confirm the LD_{50} values obtained by an acute toxicity test. Even with the HCB pretreatment, the I_{50} value can also confirm or predict the increase in the LD_{50} value for malathion due to microsomal enzyme stimulation.

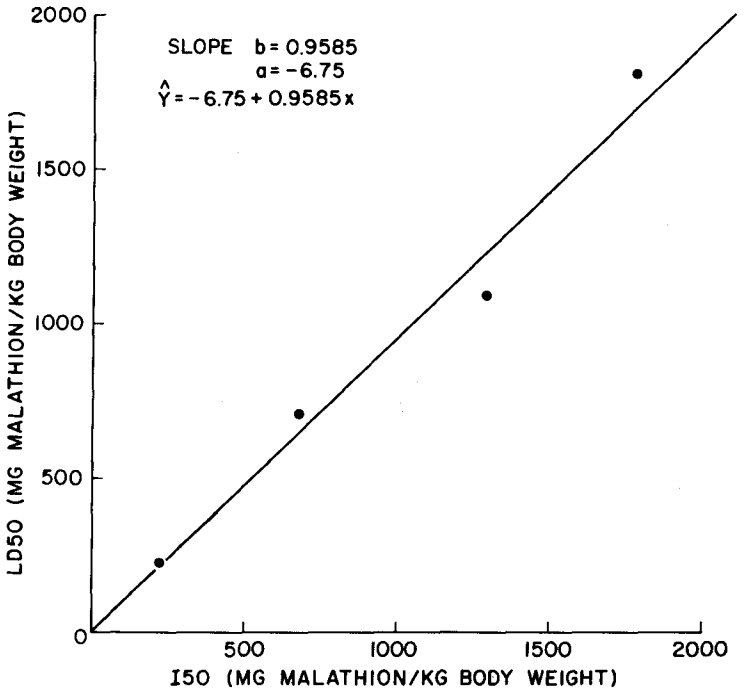

Figure 1. Relationship between LD_{50} and I_{50} for malathion in 1-, 6-, 12- and 17-day old pups (indicated by solid points o from left to right.

Figure 1 shows the relationship between the I_{50} and LD_{50} values of malathion. The sample regression coefficient $\underline{b}$ was determined to test the dependence of LD_{50} on I_{50}. The coefficient $\underline{b}$ was found equal to 0.9585 and was statistically significant at $P > 0.025$. The correlation coefficient found between I_{50} and LD_{50} was 0.9965. These data further indicate that I_{50} values of malathion based on brain acetylcholinestase inhibition can be used as reliable estimates of LD_{50}.

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