Table 2. Enzyme levels in brain cortex during ammonia toxicity

Enzyme	Control	Acute toxicity	General toxicity
GDH ($n = 6$)	0·08 ± 0·01	0·07 ± 0·01	0·06 ± 0·008
Glutamine synthetase ($n = 4$)	0·03 ± 0·004	0·03 ± 0·008	0·03 ± 0·009
Na^+-K^+ ATPase ($n = 5$)	0·06 ± 0·008	0·06 ± 0·009	0·051 ± 0·009
Pyruvate kinase ($n = 8$)	1·12 ± 0·12	1·12 ± 0·05	1·17 ± 0·11

Enzyme activity is expressed as the mean ± S.E.M. In I.U./mg protein which was measured by the method of Lowry *et al.* [8].

posal of ammonia, the brain enzymes are rarely challenged with a large ammonia load. Because of this the brain may need to be exposed to high ammonia levels for a long period of time before enzyme induction takes place. This situation is difficult to achieve in practice as we have found that there is a very narrow time margin between raising brain ammonia levels and death of the animal.

Experiments are now in progress to chronically expose the brain to slightly lower ammonia levels than shown here for longer periods of time and to monitor the ammonia metabolizing enzymes in 14 different brain regions.

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REFERENCES

1. S. Berl, *Expl. biol. Med.* **4.** 71 (1971).
2. H. Weil-Malherbe, in *Neurochemistry* (Eds. K. A. C. Elliot, I. H. Page and J. H. Quastel) p. 321. Charles C. Thomas, Springfield. (1962).
3. K. L. Reichelt, E. Kvamme and B. Tveit. *Scand. J. clin. Lab. Invest.* **16.** 433 (1964).
4. E. Schmidt, in *Methods of Enzymatic Analysis* (Ed. H. V. Bergmeyer) p. 752 (1965).
5. C. A. Woolfolk, B. Shapiro and E. R. Stadtman. *Archs Biochem. Biophys.* **116.** 177, (1966).
6. C. M. Spellman, P. F. Fottrell, S. Baynes, E. M. O'Dwyer and J. D. Clinch. *Clin. Chim. Acta* **48.** 259 (1973).
7. Boehringer Information Booklet.
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall. *J. biol. Chem.* **193.** 265 (1951).

Biochemical Pharmacology, Vol. 24, pp. 1996–1997. Pergamon Press, 1975. Printed in Great Britain.

Effect of fenitrothion on δ-aminolevulinic acid synthetase activity of mouse liver

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It is well known that numerous drugs can induce δ-aminolevulinic acid synthetase (ALA synthetase), the first enzyme in haem synthesis [1–3]. Several reports have shown that some drugs which can induce ALA synthetase decrease microsomal cytochrome P-450 content soon after their administration [4, 5]. The results suggest that these drugs may induce ALA synthetase by lowering the concentration of haem, mainly due to the loss of cytochrome P-450 in the liver, thereby decreasing the normal feed-back control.

Our early studies have revealed that some organophosphate insecticides can inhibit drug-metabolizing activity of mouse liver and that this inhibition is due to the rapid decrease in microsomal cytochrome P-450 content after the administration of fenitrothion, one of the widely used organophosphate insecticides [6].*

The results described above prompted us to determine hepatic ALA synthetase activity of mice after treatment with fenitrothion.

Male ddY mice, weighing 25–28 g and fed a commercial diet, were used. Except for the time-course study, the mice were fasted for 20 hr prior to insecticide treatment. The mice were injected intraperitoneally with different dose levels of fenitrothion dissolved in corn oil and sacrificed at the times indicated. Control mice were injected with corn oil. Livers were rapidly excised and homogenized in 3 vol. of 0·9% NaCl containing 10 mM Tris–HCl buffer (pH 7·4) and 0·5 mM EDTA using a Potter–Elvehjem homogenizer with a Teflon pestle. ALA synthetase activity was assayed as described by Marver *et al.* [7] using the total homogenate as the enzyme source. The reaction mixture contained 200 μmoles glycine, 20 μmoles EDTA, 0·4 μmole pyridoxal phosphate, 150 μmoles Tris–HCl buffer (pH 7·2), and 0·5 ml homogenate, in a final volume of 2 ml. The reaction mixture was shaken in a metabolic incubator for 60 min at 37°, and the reaction was stopped by addition of 0·5 ml of 25% trichloroacetic acid solution. The ALA produced was converted to a pyrrole by condensation with acetylacetone, and the product was isolated using a column of Dowex-1-acetate. Then, the pyrrole compound derived from ALA was determined colorimetrically by reaction with Ehrlich-Hg reagent [8].

The time-course of ALA synthetase activity after treatment with fenitrothin at 100 mg/kg dose level is shown in Fig. 1. A significant increase in ALA synthetase activity was observed shortly after the administration of fenitrothion. The peak value was reached within 4 hr and declined to the original activity within 12 hr after the administration of fenitrothion. The maximum increase in ALA synthetase

* T. Yoshida, K. Homma and M. Uchiyama, manuscript submitted for publication.

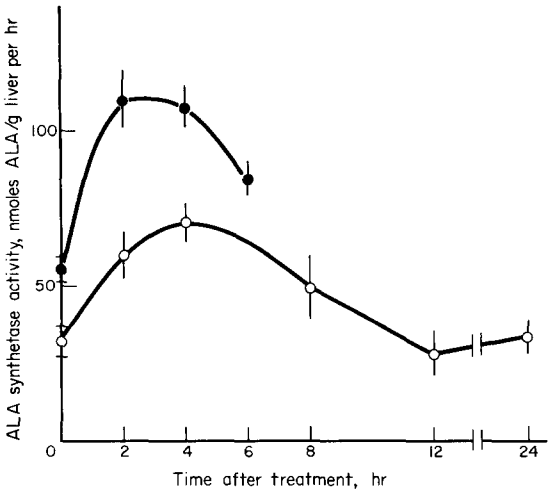

Fig. 1. Time course of ALA synthetase activity after administration of fenitrothion to fed and fasted mice. Mice were injected intraperitoneally with fenitrothion (100 mg/kg) and were sacrificed at the time indicated. Values are the mean ± S. E. of three to five mice. Key: fed (O——O); fasted (●——●).

activity was about 2·2 times the control value. In fasted mice, although the assayable activity of ALA synthetase was increased, administration of fenitrothion resulted in an effect similar to that seen in fed mice. This increase in ALA synthetase activity may have been due to an increase in new enzyme protein rather than to activation or stabilization of pre-existing enzyme, since pretreatment with cycloheximide prior to the administration of fenitrothion completely abolished the increase in ALA synthetase activity.

This transient increase in ALA synthetase activity coincides well with the decrease in cytochrome P-450 after the administration of fenitrothion in time, suggesting that the compensation for the decrease in cytochrome P-450 seems to act through a mechanism of induction of ALA synthetase.

The effect of various doses of fenitrothion on ALA synthetase activity in the liver of fasted mice is shown in Table 1.

Fenitrothion produced a dose-related increase in ALA synthetase activity in mouse liver homogenates when measured at 4 hr after administration of the insecticide. The maximum increase in ALA synthetase activity was obtained at the 200 mg/kg dose level. No further increase was observed by treatment with a dose level higher than 200 mg/kg.

Table 1. Dose–response relationship between fenitrothion and ALA synthetase activity*

Treatment	ALA synthetase activity (nmoles ALA/g liver/hr)
Control	50·9 ± 5·8
Fenitrothion	
50 mg/kg	97·9 ± 8·8
200 mg/kg	123·3 ± 13·1
300 mg/kg	116·1 ± 7·4

* Fenitrothion was administered to fasted mice 4 hr before sacrifice at the indicated dose levels. Values are the mean ± S. E. of four to five mice.

Daily administration of fenitrothion at a dose of 100 mg/kg for 3 consecutive days produced an increase in ALA synthetase activity similar to the increase following a single dose of insecticide. However, the increased ALA synthetase activity was sustained for as long as 24 hr after the last administration of fenitrothion. This result is in accordance with the observation that the repeated administration of fenitrothion to mice increased cytochrome P-450 content and drug-metabolizing activity (unpublished data).

In conclusion, a relationship appears to exist between alterations in microsomal drug-metabolizing enzyme activity and ALA synthetase activity after the administration of fenitrothion.

This study represents the first demonstration of the induction of ALA synthetase by fenitrothion. In addition, this study provides information on an effect of organophosphate insecticides which has not been previously reported.

Recent reports have shown that several organophosphate insecticides can alter microsomal drug-metabolizing activities [9–11]. Thus, this class of insecticides may, in general, exert an effect on hepatic haem synthesis.

At the present stage, however, it is not clear what kind of metabolic process or structural state is necessary for fenitrothion to bring about these interesting effects. The mechanism postulated by Norman *et al.* [12] seems the most likely explanation of the effects of fenitrothion *in vivo*. To examine this possibility, we are now carrying out investigations using carbon disulfide as a model agent, since the mechanism of its metabolism by drug-metabolizing enzyme systems appears to be similar to that of the organophosphate insecticide, parathion [13].

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REFERENCES

1. S. Granick, *J. biol. Chem.* **241**, 1359 (1966).
2. H. S. Marver, A. Collins, D. P. Tschudy and M. Rechcigl, *J. biol. Chem.* **241**, 4323 (1966).
3. F. DeMatteis, *Pharmac. Rev.* **19**, 523 (1967).
4. O. Wada, Y. Yano, G. Urata and K. Nakao, *Biochem. Pharmac.* **17**, 595 (1968).
5. F. De Matteis, *Biochem. J.* **124**, 767 (1971).
6. M. Uchiyama, T. Yoshida, K. Homma and T. Hongo, *Biochem. Pharmac.* **24**, 122 (1975).
7. H. S. Marver, D. P. Tschudy, M. G. Perlroth and A. Collins *J. biol. Chem.* **241**, 2803 (1966).
8. G. Urata and S. Granick, *J. biol. Chem.* **238**, 811 (1963).
9. J. T. Stevens, *Life Sci.* **14**, 2215 (1974).
10. J. T. Stevens and F. E. Greene, *Bull. envir. Contam. Toxic.* **11**, 538 (1974).
11. R. P. Weber, J. M. Coon and A. J. Triolo, *Cancer Res.* **34**, 947 (1974).
12. B. J. Norman, R. E. Poore and R. A. Neal, *Biochem. Pharmac.* **23**, 1733 (1974).
13. R. R. Dalvi, R. E. Poore and R. A. Neal, *Life Sci.* **14**, 1785 (1974).