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Phenobarbital-induced enzymatic and non-enzymatic lipid peroxidation in rat liver microsomes*

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Our laboratory has recently shown that phenobarbital (PB) administration produced triglyceride accumulation in rat liver [1, 2]. Since peroxidative decomposition of hepatic polyunsaturated lipids is currently believed to be a mechanism responsible for the development of a fatty liver and cell necrosis [3, 4], we have investigated the effect of PB on hepatic lipid peroxidation. In a recent report [5], we have established that PB enhances lipid peroxidation in the liver as reflected by increased hepatic malonic dialdehyde (MDA) concentrations and diene conjugation.

Oxidative degradation or peroxidation of phospholipids in hepatic microsomes is produced by two different systems. One of these is an NADPH-dependent enzyme which involves ADP and the reduction of Fe^{3+} to Fe^{2+} [6]. The other is a non-enzymatic, ascorbate-dependent reaction [7]. Since it has been established that PB elevates hepatic lipid peroxidation, the present study was conducted to determine whether this drug induces hepatic microsomal enzymatic (NADPH-dependent) lipid peroxidation or non-enzymatic (ascorbate-dependent) lipid peroxidation or both.

Male Sprague–Dawley rats (250 g) were maintained on a synthetic diet as previously described [1]. This diet contained 35% fat, 24% protein and 2.1% choline dihydrogen citrate. One group of rats was injected intraperitoneally with 50 mg/kg body weight of PB every 12 hr for 5 days. Control rats received injections of equal volumes of saline. Rats in the control and PB groups were pair-fed and given water *ad lib*. The animals were killed by decapitation and their livers were removed and weighed. The fresh liver was homogenized in 0.174 M KCl, 0.25 M Tris–HCl buffer at pH 7.4 according to Hunter *et al.* [8]. Sucrose was avoided, since it has been reported to interfere with MDA determinations [9]. Microsomes from liver homogenate were prepared according to the method of Noguchi *et al.* [10].

Since it was shown in an earlier report [5] that enhanced hepatic lipid peroxidation induced by PB could be demonstrated either with MDA production or diene conjugation, lipid peroxidation in this study was measured as MDA production according to Tappel and Zalkin [11]. Previous investigators have shown [12–15] that the cofactor complex Fe^{2+} + ADP is essential for maximum lipid peroxidation. These cofactors in the presence of adequate reducing agents were added to both control and PB-treated homogenates in the following manner: Fe^{2+} (2 μM) + ADP (0.5 mM) + NADPH (0.5 mM) and Fe^{2+} (2 μM) + ADP (0.5 mM) + ascorbate (0.5 mM). Enzymatic peroxidation in the microsomes was determined according to a modification of the incubation procedures of Fukuzawa and Uchiyama [12]. The reaction mixture contained 0.5 ml of microsomal suspension and the total volume was adjusted to 4 ml with phosphate buffer at pH 7.4. This mixture was incubated at 37°C for 30 min. Non-enzymatic lipid peroxidation was performed under the same conditions except that the microsomes were boiled for 5 min to inactivate microsomal enzymes.

In agreement with our previous report [5], hepatic microsomes of PB-treated animals in this study (Table 1) showed an increased capacity for enzymatic lipid peroxidation when compared to controls. In control microsomes and microsomes from PB-treated animals, enzymatic lipid peroxidation was increased significantly upon the addition of Fe^{2+} and ADP with either NADPH or ascorbate. In each case, however, the microsomes from PB-treated animals contained more peroxidative activity than the microsomes from untreated animals.

As shown in Table 1, the lipid peroxidation of non-enzymatic microsomes was also increased by PB administration. Ascorbate but not NADPH further stimulated control was well as PB-induced lipid peroxidation.

An NADPH-dependent lipid peroxidation system was first described in rat liver microsomes by Hochstein *et al.* [14, 16]. Recent publications [17–25] have suggested that cytochrome *c* reductase (EC 1.6.99.3) is the enzyme which mediates lipid peroxidation, and it has been postulated that NADPH via this enzyme maintains the iron of the Fe^{2+} + ADP complex in the reduced state [15].

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Table 1. Effect of phenobarbital (PB) on the NADPH-linked enzymatic and ascorbate-linked non-enzymatic lipid peroxidation in rat liver microsomes (μg MDA produced/mg protein)*

	Enzymatic			Non-enzymatic		
Supplementation	Control (8)†	PB-treated (8)†	P‡	Control (8)†	PB-treated (8)†	P‡
Nil	0.06 ± 0.01	0.11 ± 0.03	< 0.01	0.09 ± 0.02	0.21 ± 0.04	< 0.001
Fe^{2+} + ADP + NADPH	1.69 ± 0.29§	2.43 ± 0.20§	< 0.001	0.11 ± 0.02‖	0.27 ± 0.04¶	< 0.001
Fe^{2+} + ADP + Ascorbate	1.91 ± 0.26§	2.37 ± 0.35§	< 0.025	1.37 ± 0.14§	1.55 ± 0.11§	< 0.025

* Mean values ± S. D.
† Number of animals.
‡ PB-treated compared to controls
§ P < 0.001 compared to nil supplementation.
‖ Not significant compared to nil supplementation.
¶ P < 0.01 compared to nil supplementation.

This study demonstrates that hepatic microsomes from PB-treated animals, whether stimulated with cofactors or not, exhibit increased enzymatic peroxidative activity when compared to controls. This is in agreement with previous observations [26] that PB is an inducer of microsomal cytochrome *c* reductase (EC 1.6.99.3).

These data further demonstrate that non-enzymatic lipid peroxidation, whether stimulated by cofactors or not, is also increased by PB. The explanation for this increased activity is not readily apparent. It is possible that the PB-induced increase in microsomal phospholipid [27, 28], with its resultant high level of unsaturated fatty acids, could furnish increased available substrate for lipid peroxidation.

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REFERENCES

1. M. F. Sorrell, D. J. Tuma and A. J. Barak, *Proc. Soc. exp. Biol. Med.* **143,** 839 (1973).
2. D. J. Tuma, M. F. Sorrell and A. J. Barak, *Proc. Soc. exp. Biol. Med.* **146,** 953 (1974).
3. R. O. Recknagel, E. A. Glende, Jr., G. Ugazio, R. R. Koch and S. Srinivason, *Israel J. med. Sci.* **10,** 301 (1974).
4. N. R. DeLuzio, *Fedn. Proc.* **32,** 1875 (1973).
5. H. K. J. Hahn, D. J. Tuma, A. J. Barak and M. F. Sorrell, *Biochem. Pharmac.* **25,** 769 (1976).
6. A. Beloff-Chain, G. Serlupi-Crescenzi, R. Catanzara, D. Venettaci and M. Balliano, *Biochim. biophys. Acta* **97,** 416 (1965).
7. A. Ottlenghi, *Archs. Biochem. Biophys.* **79,** 355 (1957).
8. F. E. Hunter, Jr., A. Scott, P. E. Hoffstein, J. M. Gelichi, J. Weinstein and A. Schneider, *J. biol. Chem.* **239,** 614 (1964).
9. L. Ernster and K. Nordenbrand, *Meth. Enzym.* **10,** 574 (1967).
10. T. Noguchi, H. Cantor and M. L. Scott, *J. Nutr.* **103,** 1502 (1973).
11. A. L. Tappel and H. Zalkin, *Archs. Biochem. Biophys.* **70,** 326 (1959).
12. K. Fukuzawa and M. Uchiyama, *J. Nutr. Sci. Vitamin.* **19,** 433 (1973).
13. A. K. Schneider, E. E. Smith and F. E. Hunter, Jr., *Bio-chemistry* **3,** 1470 (1964).
14. P. Hochstein and L. Ernster, *Biochem. biophys. Res. Commun.* **12,** 388 (1963).
15. M. Nakano, H. Fukuzawa, K. Suwa and Y. Tsutsumi, *Proc. Soc. exp. Biol. Med.* **144,** 164 (1973).
16. P. Hochstein, K. Nordenbrand and L. Ernster, *Biochem. biophys. Res. Commun.* **114,** 323 (1964).
17. E. D. Wills, *Biochem. J.* **113,** 315 (1969).
18. E. D. Wills, *Biochem. J.* **113,** 325 (1969).
19. E. D. Wills, *Biochem. J.* **113,** 333 (1969).
20. H. E. May and P. B. McCay, *J. biol. Chem.* **243,** 2288 (1968).
21. H. E. May and P. B. McCay, *J. biol. Chem.* **243,** 2296 (1968).
22. B. K. Tam and P. B. McCay, *J. biol. Chem.* **245,** 2295 (1970).
23. J. L. Poyer and P. B. McCay, *J. biol. Chem.* **246,** 263 (1971).
24. P. M. Pfeifer and P. B. McCay, *J. biol. Chem.* **246,** 6401 (1971).
25. P. B. McCay, J. L. Poyer and P. M. Pfeifer, *Lipids* **6,** 297 (1971).
26. A. H. Conney, *Pharmac. Rev.* **19,** 317 (1967).
27. B. B. Brodie, J. R. Gillette and B. N. LaDu, *A. Rev. Biochem.* **27,** 427 (1958).
28. D. L. Young, G. Powell and W. O. McMillan, *J. Lipid Res.* **12,** 1 (1971).

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