EFFECT OF PROTEIN-CALORIE MALNUTRITION ON DRUG METABOLISING ENZYMES IN RAT LIVER

A. P. S. NARANG*

Department of Hepatology,
Postgraduate Institute of Medical Education and Research,
Chandigarh - 160 012

(Received on April 2, 1987)

Summary : Drugs are chiefly metabolished in the liver usually in two phases, viz. oxidation and conjugation. The present study was undertaken to investigate the effect of protein-calorie malnutrition (PCM), rehabilitation and effect of phenobarbitone on the hepatic drug metabolising enzymes in weanling albino rats, fed on a semisynthetic diet containing 18% or 0.5% protein. The two representative enzymes of oxidation and conjugation employed were aminopyrine N-demethylase and bilirubin UDP-glucuronyl transferase, respectively. The study revealed that PCM severely impairs oxidative drug metabolising enzyme but less so in conjugation stage. On refeeding 18% protein diet, drug metabolising enzymes returned to normal within 2-3 weeks. Phenobarbitone administration increased the activities of drug metabolising enzymes.

Key words : protein calorie malnutrition, drug metabolising enzymes, aminopyrine N-demethylase, bilirubin UDP-glucuronyl transferase

INTRODUCTION

Protein calorie — malnutrition (PCM) and its cures are of great interest as one - third of the children from under developed countries suffer from some degree of malnutrition (43). It is known that protein % deficiency causes a decrease in hepatic protein % content (26). There is reduced incorporation of amino acids into protein by cell-free preparations of livers (21). It has been reported that starving male mice for 36 hours resulted in a marked decrease in the hepatic metabolism of several drugs (8). A deficiency of dietary protein was to increase the toxicity of aflatoxin for rats (20).

*Present address : Deparment of Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences, Srinagar - 190 011

More than 200 drugs, insecticides, carcinogens, and other chemicals are known to stimulate drug metabolism when administered to laboratory animals (22). Phenobarbitone is a prime example of a drugs that has a direct effect of enzyme induction (18, 37). Other important inducers of drug metabolism are : alcohol (39), chloral hydrate (46), glutethimide (11), qninine (2), ether (41), ketamine (23), polychlorinated dibenzofurane (17), acetone (7) and 3-methylcholanthrene (45). Drug metabolism can be inhibitated by disulfiram (12), proprenolol (15), carbon monoxide (25), zinc (6), cyanide (19), lead (13), galactosamine (44), arachidonic acid (38) obstructive jaundice (27) and liver disease (28-35). A kwashiorkor like syndrome has been induced in growing rats fed on a diet containing 0.5% lactalbumin (9). Drug clearance has been reported to be slow in children suffering from PCM (24, 36).

It is known that majority of lipid soluble drugs are metabolised in liver and involves a two step process, viz. oxidation and conjugation. A reduction in oxidative drug metabolising enzymes in rats following PCM has been observed (4) and on refeeding they restore these enzymes to normal level (5). On the other hand, no reduction was observed in conjugating enzymes (47). It was thought worth while to investigate the effect of PCM on oxidative and conjugating enzymes together in weanling rats. The two representative enzymes for oxidation and conjugation used in the present study were aminopyrine N-demethylase and bilirubin UDP-glucuronyl transferase.

MATERIAL AND METHODS

Two hundred male rats of Wistar strain weighing 30-40 *g* were utilised for the present study. They were divided into two groups. One group was fed on 18% protein (casein) diet throughout and served as controls. Second group of rats were fed on 0.5% casein diet for two weeks. Then they were divided into different groups. One group of rats was sacrificed alongwith same number of controls. Another group was administered phenobarbitone intraperitoneally for seven days (1) alongwith control group. The dosage of phenobarbitone were : 20 *mg/kg* body wt. for 2 days, 40 *mg/kg* body wt. for 2 days, 60 *mg/kg* body wt. for 2 days and 80 *mg/kg* body wt. for 1 day. The other groups received 18% casein diet upto the time of sacrifice. Each rat was given diet at the rate of 10 *g*/100 body weight. Tap water was given freely. The effect of protein refeeding on drug metabolising enzymes at 1, 2, 3, 4, 5 and 6 weeks were investigated. Ten rats were sacrificed in each group alongwith similar number of controls.

Rats fasted overnight were anaesthetised by anesthetic ether. The abdominal wal was opened and blood drawn from the inferior vena cava. The total plasma protein con centration was measured by the biuret method (14). The livers were quickly removed

blotted and weighed. Aminopyrine N-demethylase was determined by measuring the formation of formaldehyde (42). Bilirubin UDP-glucuronyl transferase was determined from the amount of direct reacting bilirubin in the incubation medium (3). Liver proteins were measured by biuret method (14).

RESULTS

After two weeks of low protein diet, rats lost their body weight by 54% as compared to controls. When they were refed on 18% protein diet, after one week, they started gaining weight very rapidly. After six weeks, there was no significant difference between the two groups. PCM significantly decreased liver weight (Table I). However,

TABLE I : Effect of protein - calorie malnutrition, rehabilitation and phenobarbitone on liver weight, liver and plasma protein (The number of rats sacrificed in each group is ten).

Group	*Liver Wt. (g)*	*Liver Wt.% (Body Wt.)*	*Liver Proteins (mg/g)*	*Plasma Proteins (g/100 ml)*
Controls (18 protein for two weeks)	2.28±0.17	4.64±0.38	250±5.96	7.50±0.10
Malnourished (0.5% protein for two weeks)	1.39±0.10 (P<0.001)	5.79±0.80 (P>0.05)	192±6.82 (P<0.001)	3.80±0.38 (P<0.001)
Controls	3.07±0.31	4.61±0.52	243±6.91	7.43±0.15
Malnourished + Refeeding for one week	1.77±0.18 (P<0.005)	4.54±0.56 (P>0.05)	195±4.71 (P<0.001)	4.99±0.45 (P<0.001)
Controls	3.92±0.35	4.81±0.35	247±9.53	7.47±0.32
Malnourished + Refeeding for two week	2.80±0.21 (P<0.02)	4.59±0.21 (P>0.05)	209±7.05 (P<0.005)	5.62±0.36 (P<0.005)
Controls	4.87±0.52	4.58±0.39	251±5.67	7.52±0.19
Maluourished + Refeeding for three weeks	3.01±0.43 (P<0.02)	4.18±0.57 (P>0.05)	218±2.31 (P<0.001)	6.25±0.30 (P<0.005)
Controls	5.39±0.27	4.54±0.43	249±4.98	7.44±0.23
Malnourished + Refeeding for four weeks	3.40±0.42 (P<0.001)	4.19±0.53 (P>0.05)	233±2.31 (P<0.01)	7 19±0.28 (P<0.05)
Controls	6.53±0.23	4.53±0.38	252±8.16	7.39±0.41
Malnourished + Refeeding for five weeks	5.80±0.24 (P<0.001)	4.29±0.30 (P>0.05)	242±6.16 (P>0.05)	7.20±0.32 (P>0.05)
Controls	6.80±0.11	4.43±0.10	255±5.72	7.50±0.24
Malnourised + Refeeding for six weeks	5.90±0.15 (P<0.001)	4.22±0.21 (P>0.05)	243±3.11 (P>0.05)	7.30±0.24 (P>0.05)
Controls + Phenobarbitone	5.23±0.21	6.54±0.53	276±4.79	7.45±0.23
Malnourished + Phenobarbitone	3.59±0.15 (1<0.001)	5.96±0.35 (P>0.05)	208±6.31 (P<0.001)	4.93±0.34 (P<0.001)

Results are expressed as means ± S.E.

there was no change in terms of liver weight percentage of body weight. There was a significant decrease of plasma proteins in malnourished rats, refeeding of 18% protein diet brought it to normal level after four weeks. The protein content of the livers of malnourished animals were decreased significantly (Table I).

Table II depicts the effect of malnutrition of aminopyrine N-demethylase and bilirubin UDP-glucuronyl transferase. Oxidative enzyme, aminopyrine N-demethylase was markedly reduced by PCM ($P<0.001$). On refeeding, the enzyme activity increased. After three weeks of rehabilitation, the activity was in the normal range. Similarly, bilirubin UDP-glucuronyl transferase decreased significantly and increased on refeeding.

TABLE II : Effect of protein — calorie malnutrition, rehabilitation and phenobarbitone on drug metabolising enzymes (Ten rats were killed in each group).

Group	*Aminopyrine N-demethylase (nmol/min/g liver)*	*Bilirubin uDP-glucuronyl transferase (µmol/hr/g liver)*
Controls	77.0±2.79	1.80±0.120
Malnourished	23.3±1.36	1.20±0.096
	(P<0.001)	(P<0.005)
Controls	75.5±3.32	1,82±0.134
Malnourished +	46.0±1.12	1.22±0.075
Refeeding for one week	(P<0.001)	(P<0.005)
Controls	79.3±2.33	1.95±0.152
Malnourished +	50.5±1.66	1.31±0.047
Refeeding for two weeks	(P<0.001)	(P<0.001)
Controls	73.4±2.57	1.72±0.079
Malnourished +	67.6±2.28	1.41±0.055
Refeeding for three weeks	(P>0.05)	(P<0.01)
Controls	82.7±4.31	1.81±0.162
Malnourished +	76.2±3.50	1.63±0.062
Refeeding for four weeks	(P>0.05)	(P>0.05)
Controls	80.3±2.39	1.93±0.105
Malnourished +	75.5±2.31	1.86±0.050
Refeeding for five weeks	(P>0.05)	(P>0 05)
Controls	83.0±2.55	2.17±0.208
Malnourished +	76.0±3.69	2.01±0.163
Refeeding for six weeks	(P>0.05)	(P>0.05)
Controls + Phenobarbitone	106.0±6.95	3.36±0.170
Malnourished + Phenobarbitone	68.0±2.83	1.63±0.191
	(P<0.001)	(P<0.001)

Results are expressed as means ± S.E.

Rats treated with phenobarbitone for one week had a significantly increase of liver proteins. There was no significant change in plasma proteins. An increase in aminopyrine N-demethylase and bilirubin UDP-glucuronyl transferase was observed following phenobarbitone administration.

DISCUSSION

It is evident from the study that 14 days of protein deficiency in young rats promptly resulted in growth retardation. It is shown that malnutrition have a profound effect on drug metabolising enzymes especially aminopyrine N-demethylase. This could be due to the alteration in Km caused by dietary protein deficiency either through the deficiency of apoenzymes or the membrane protein which is integral part of the drug metabolising system. Dietary protein deficiency could also lead to a more indirect effect, either through an alteration of the synthesis or breakdown of the components like phospholipids which are required for the normal enzyme activities. It has been shown (40) that 3% casein diet for 14 days in rats cause significant reduction in cytochrome P-450 level which is involved in the oxygen activation and substrate binding prior to the oxidation of substrate. The low activity of aminopyrine N-demethylase system which parallels the cytochrome P-450, is quite indicative of this phenomenon. However Anthony observed that inspite of normal level of cytochrome P-450, % N-demethylation can be markedly reduced in malnourished rats (1), suggesting thereby that these components may not be exclusive determining factors of phase I reaction of foreign compounds. Remmer presented evidence for the involvement of a substrate binding protein for the oxidation reaction (40). It is possible that in malnourished animals this additional factor is relatively affected by protein deprivation. The observation of disruption of polyribosemes leading to the diminished rate of ribosomal protein synthesis in malnourished rats favour this postulate (10).

In the present study, hepatic bilirubin UDP-glucuronyl transferase was also found to be decreased by malnutrition. An increase was observed in glucuronyl transferase activity after feeding protein free diet to mature rats (47). In weanling rats which were fed 0.5% protein diet, significant reduction of the enzyme was observed. It is possible that in developing stage, the enzyme activity is affected by malnutrition and not in adult animals.

Refeeding for six weeks resulted in the gain of enzymes activity and liver proteins. As results indicated three week of refeeding normal protein diet brought the enzyme level comparable to that of controls.

Administration of phenobarbitone produced induction of both drug metabolizing enzymes. It has been reported that treatment of animals with phenobarbital cause rises in the enzyme activity in microsomes (40). When rats treated with phenobarbitone, there can be a three-to four-fold increase in the microsomal content of cytochrome P-450 and a two fold increase in the reductase, and drugs such as methadone and ethylmorphine are metabolised three or four times as fast (16). Phenobarbitone is able to increase the drug metabolising capacity per unit of mass, without causing an increase in total liver size. Due to increase in endoplasmic reticulum, phenobarbitone was able to increase enzymes activities in controls as well as malnourished rats.

REFERENCES

1. Anthony, L.E. Effects of protein — calorie malnutrition on drug metabolism in rat liver microsomes. *J. Nutr.*, **103** : 811-820, 1973.
2. Berlin, C.M., J.M. Stackman and E.S. Vesell. Quinine-induced alterations in drug disposition. *Clin. Pharmac. Ther.*, **18** : 670-679, 1975.
3. Black, M., B.H. Billing and K.P.M. Heirwegh. Determination of bilirubin uDP-glucuronyl transferase activity in needle biopsy specimens of human liver. *Clin. Chim. Acta.*, **29** : 27-35, 1970.
4. Campbell, T.C. and J.R. Hayes. Role of nutrition in the drug metabolizing enzyme system. *Pharmacol. Rev.*, **26** : 171-197, 1974.
5. Campbell, T.C. and J.R. Hayes. The effect of quantity and quality of dietary protein on drug metabolism. *Fed. Proc.*, **35** : 2470-2474, 1976.
6. Chvapil, M., I.G. Sipes, J.D. Ludwig and S.V. Halladay. Inhibition of NADPH oxidation and oxidative metabolism of drugs in liver microsomes by zinc, *Biochem. Pharmac.*, **24** : 917-918, 1975.
7. Clark, H. and G. Powis. Effect of acetone administered *in vivo* upon hepatic microsomal drug metobolising activity in the rat. *Biochem. Pharmac.*, **23** : 1015-1019, 1974.
8. Dixon, R.L., R.W. Shultice and J.R. Foutes. Factors affecting drug metabolism by liver microsomes. IV. Starvation, *Proc. Soc. Exp. Biol. Med.*, **103** : 333-335, 1960.
9. Edozien, J.C. Experimental Kwashiorkor and marasmus. *Nature*, **220** : 917-919, 1968.
10. Enwonwu, C.O. and L.M. Sreebny. Studies of hepatic lesions of experimental protein — calorie malnutrition in rats and immediate effects of refeeding an adequate protein diet. *J. Nutr.*, **101** : 501-516, 1971.
11. Farell, G C., W.G.E. Cooksley and L.W. Powell. Enhancement of hepatic drug metabolism by glutethimide in patients with liver disease. *Eur. J. Clin. Pharmac.*, **16** : 113-118, 1979.
12. Freundt, K.J. Variable inhibition of human hepatic drug metabolizing enzymes by disulfiram. *Int. J. Clin. Pharmac. Biopharm.*, **16** : 323-330, 1978.
13. Goldberg, A., P.A. Meredith, S. Miller, M.R. Moore and G.G. Thompson. Hepatic drug metalbolism and haem biosynthesis in lead poisoned rats. *Br. J. Pharmac.*, **62** : 529-536, 1978.
14. Gornall, A.G., G.L. Bradawill and M.M. David. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.*, **177** : 751-766, 1949.

15. Greenblatt, D.J., K. Frank and D.H. Huffman. Impairment of antipyrine clearance in humans by propranolol. *Circulation.* **57** : 1161-1164, 1978.

16. Kappas, A. and A.P. Alvares. How the liver metabolizes foreign compounds? *Sci. Am.*, **232** : 22-31, 1975.

17. Kawano, S. and K. Hiraga. Polychlorinated dibenzo-furans-Potent inducers of rat hepatic drug metabolising enzymes. *Jap. J. Pharmac.*. **28** : 305-315, 1978.

18. Kellermann, G. and M. Luyten-Kellermann. Phenobarbital induced drug metabolism in man. *Tox. App. Pharmac.*, **39** : 97-104, 1977.

19. Kitada, M., K. Chiba, T. Kamatoki and H. Kitagawa. Inhibition of ayanide by drug oxidation in rat liver microsomes. *Jap. J. Pharmac.*, **27** : 601-608, 1977.

20. Madhavan, T.V. and C. Gopalan. Effect of dietary protein on aflatoxin liver injury in weanling rats. *Arch. Path.*, **80** : 123-126, 1965.

21. Mandal, P., C. Quirin, M. Block and M. Jacob. The influence of protein intake on RNA and protein synthesis in rat liver. *Life. Sci.*, **5** : 325-330, 1966.

22. Mannering, G.J. Significance of stimulation and inhibition of drug metabolism in pharmacological testing. In Selected Pharmacological Testing Methods — Ed, A. Burger Marcel Decker, New York, p. 51, 1968.

23. Marietta, M.P., M.E. Vore, E.L. Way and A.J. Trevor. Characterization of ketamine induction of hepatic microsomal drug metabolism. *Biochem. Pharmac.*, **26** : 2451-2453, 1977.

24. Mehta, S., H.K. Kalsi, S. Jayaraman and V.S. Mathur. Chloramphenicol metabolism in children with protein-calorie malnutrition. *Am. J. Clin. Nutr.*, **28** : 977-981, 1975.

25. Montgomery, M.R. and R.J. Rubin. Oxygenation during inhibition of drug metabolism by carbon dioxide or hypoxic hyposia. *J. Appl. Physiol.*, **35** : 505-509, 1973.

26. Munro, H.N. Mammalian protein metabolism. Vol I, eds. H.N. Munro and J B. Allison. Academic Press, New York, p. 381, 1964.

27. Narang, A.P.S., C.R. Nair, R. Nair and D.V. Datta. Effect of low protein diet on drug metabolising enzymes in common bile duct ligation. *J. Assoc. Phys. India.*, **26** : 155-158, 1978.

28. Narang, A.P.S., V.S. Mathur, A. Koshy, N. Nath and D.V. Datta. Drug metabolism in non-cirrhotic portal fibrosis and other liver disease. *Ind. J Med. Res.*, **74** : 266-272, 1981.

29. Narang, A.P.S., D.V. Datta, N. Nath and V.S. Mathur. Pharmacokinetic study of chloramphenicol in patients with liver disease. *Eur. J. Clin. Pharmac.*, **20** : 479-483, 1981.

30. Narang, A.P.S., D.V. Datta and V.S. Mathur. Impairment hepatic drug metabolism in patients with Budd-Chiari syndrome. *Indian. J. Med. Res.*, **75** : 567-573, 1982.

31. Narang, A.P.S., D.V. Datta and V.S. Mathur. Antipyrine clearance, aminopyrine N-demethylase and bilirubin uDP-glucuronyl transferase activity in patients with amoebic liver abscess. *Biopharm. Drug Dispos.*, **3** : 39-45, 1982.

32. Narang, A.P.S. and D. V. Datta. Comparison of *in vivo* and *in vitro* drug metabolism in patients with Gilbert's syndrome. *J. Assoc. Phys. India.*, **30** : 357-359, 1982.

33. Narang, A.P.S. D.V. Datta, N. Nath and V.S. Mathur. Impairment of hepatic drug metabolism in patients with acute viral hepatitis. *Europ. J. Drug. Metab. Pharm.*, **7** : 255-259, 1982.

34. Narang, A.P.S., D.V. Datta and V.S. Mathur. *In vitro* drug metabolism in man with different liver diseases. *Int. J. Clin. Pharmac. Ther. Toxicol.*, **21** : 496-498, 1983.

35. Narang, A.P.S., D.V. Datta and V.S. Mathur. Impairment of drug elimination in patients with liver disease. *Int. J. Clin. Pharmac. Ther. Toxicol.*, **23** : 28-32, 1985.

36. Narang, R.K., S. Mehta and V.S. Mathur. Pharmacokinetic study of antipyrine in malnourished children. *Am. J. Clin. Nutr.*, **30** : 1979-1982, 1977.

37. Negishi, M., Y. Fujii-Kuriyama, Y. Tashiro and Y. Imai. Site of biosynthesis of cytochrome P-450 in hepatocytes of phenobarbital treated rats. *Biochem. Biophys. Res. Commun.*, **71** : 1153-1160, 1976.

38. Pessayre, D.P. Mazel, V. Descatoire, E. Rogier, G. Feldmann and J. P. Benhamou. Inhibition of hepatic drug metabolising enzymes by arachidonic acid. *Xenobiotica.*, **9** : 301-306, 1979.

39. Pirttiaho, H.I., E.A. Sotaniemi, J. Ahlqvist, U. Pitkanen and R.O. Pelkonen. Liver size and indices of drug metabolism in alcoholics. *Eur. J. Clin. Pharmac.*, **13** : 61-67, 1978.

40. Remmer, H., J.B. Schenkmann and H. Greim. Microsomes and drug oxidation. eds. J.R. Gillete, A.H. Conney, G.J. Cornides, R.W. Estabrook, J.R. Fouts and G.J. Mannering. Academic Press, New York, p. 371, 1969.

41. Ross, W.T. and R.R. Cardell. Proliferation of smooth endoplasmic reticulum and induction of microsomal drug metabolising enzymes after ether or halothane. *Anesthesiology*, **48** : 325-331, 1978.

42. Schoene, B., R.A. Fleischmann, H. Remmer and H.F. V. Older-hausen. Determination of drug metabolizing enzymes in needle biopsies of human liver. *Eur. J. Clin. Pharmacol.*, **4** : 65-73, 1972.

43. Scrimshaw, N.S., C.E. Teylor and I.E. Gorden, World Health Organisation. Monger, Sr. No. 57, p. 329, 1968.

44. Shigeta, Y. Microsomal drug metabolism in acute and chronic galactosamine induced hepatic injuries. *Gastroent. Jap.*, **12** : 435-445, 1977.

45. Sweeney, G.D., K.G. Jones and F. Krestynski. Effects of phenobarbital and 3-methyl cholanthrene pretreatment on size sedimentation velocity and mixed function oxygenase activity of rat hepatocytes. *J. Lab. Clin. Med.*, **91** : 444-454 1978.

46. Weiner, M. Species differences in the effect of chloral hydrate on coumarin anticoagulant. *Ann. N.Y. Acad. Sci.*, **179** : 226-234, 1971.

47. Woodcock, B.G. and G.C. Wood. Effect of protein free diet on uDP — glucuronyl transferase and sulphotransferase activities in rat liver. *Biochem. Pharmac.*, **20** : 2703-2713, 1971.