4. E. Hüttemann, D. Ukena, V. Lenschow, et al., Naunyn-Schmiedeberg's Arch. Pharmacol., **325**, 226 (1984).
5. J. R. Jefferson, J. T. Harmon, and G. Jamieson, Blood, **71**, 110 (1988).
6. R. N. Kalaria and S. I. Haric, J. Cereb. Blood Flow Metab., **8**, 31 (1988).
7. C. P. Page, TIPS, **9**, 66 (1988).
8. F. N. Valone, Thromb. Res., **50**, 103 (1988).
9. E. Vinge, K.-E. Andersson, and C. G. A. Persson, Acta Physiol. Scand., **120**, 117 (1984).
10. A. D. Zurn and K. Q. Do, Proc. Nat. Acad. Sci. USA, **85**, 8301 (1988).

INDUCTION OF THE MONOOXYGENASE SYSTEM AND INCORPORATION OF RADIOACTIVITY FROM 2-^{14}C-LYSINE INTO HEPATIC MICROSOMES OF PHENOBARBITAL-TREATED RATS FED A DIET DEFICIENT IN LYSINE, METHIONINE, THREONINE, AND VITAMINS A, C, AND E

T. Zh. Nurmagambetov, B. B. Amirov, T. K. Kuanysheva, and T. Sh. Sharmanov

UDC 616.36-008.931:577.152.1]-02:615.214.22]-092.9-07

KEY WORDS: monooxygenase system; rat liver; nutrition; phenobarbital

Induction of the cytochrome P-450-dependent microsomal monooxygenase system of the mammalian liver by phenobarbital is known to take place through enhancement of protein synthesis de novo [1]. In this connection, it is logical to suggest on nutritional grounds that under conditions of deficiency of essential components of the diet, the induction of this enzyme system as an adaptive response to those xenobiotics that are inducers of phenobarbital type, may lead not only to redistribution of the flow of nutrients entering with the diet, but also to the outflow of deficient essential components into the liver from other organs and tissues, which could lead to damage to the other functions of the body and could thereby aggravate the manifestations of multiple nutrient deficiency.

To test this hypothesis experimentally we studied induction of the monooxygenase system and incorporation of 2-^{14}C-lysine into microsomal proteins and certain other fractions of liver homogenate under the influence of phenobarbital and in animals with a balanced diet and with a diet deficient in three essential amino acids, namely lysine, methionine, and threonine, and also of vitamins A, C, and E.

EXPERIMENTAL METHOD

Experiments were carried out on 16 growing male WAG rats weighing initially 40-60 g, rising to 160-200 g at the time of sacrifice. The animals, divided into two groups with eight rats in each group, were kept initially for 2 months on a balanced diet (group 1) or on a diet deficient in lysine, methionine, threonine, and vitamins A, C, and E (group 2). The composition of the diets was given previously [4]. In each group the animals were divided into two subgroups: the experimental rats were given phenobarbital (PB, from "Merck," Germany) intraperitoneally for 3 days in a dose of 80 mg/kg, in the form of a sterile solution in 0.9% NaCl, whereas the control rats were given the solvent

Kazakh Branch, Institute of Nutrition, Russian Academy of Medical Sciences, Alma-Ata. (Presented by Academician of the Russian Academy of Medical Sciences A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 609-612, June, 1992. Original article submitted September 20, 1991.

0007-4888/92/0006-0813$12.50

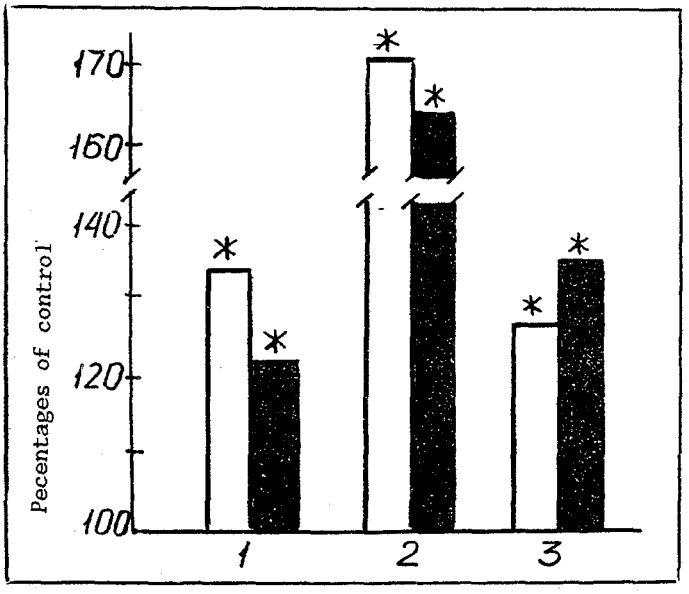

Fig. 1. Effect of three injections of phenobarbital in a dose of 80 mg/kg against a background of different diets on microsomal protein concentration and relative mass of rat liver. Unshaded columns – balanced diet, black columns – diet deficient in lysine, methionine, and threonine and in vitamins A, C, and E. 1) Content of microsomal protein per gram liver, 2 and 3) content of microsomal protein and mass of liver per 100 g body weight respectively. Values of corresponding parameters in control animals taken as 100%. Asterisks indicate parameters differing significantly from control ($p < 0.05$).

only. All the animals were given fractional intraperitoneal injections (4 times every 4 h) of 2-^{14}C-lysine ("Izotop," USSR) in a total dose of 80 μCi/100 g body weight. The quantity of labeled lysine injected was 180 times less than the quantity necessary to meet the daily deficit in the animals of group 2.

After the last injection of PB the animals were deprived of food and were decapitated 24 h later. The liver was perfused with cold 0.9% NaCl solution and the microsomal fraction was isolated [6]. To determine the level of radioactivity in the microsomal preparations, in the cytosol, and in remnants of the cells after centrifugation of liver homogenates at 10,000g, and in homogenates of the femoral muscle of the rats, aliquots of suspensions of the corresponding biological materials, containing 4 mg protein, were added to 2 ml of 10% TCA and filtered through nitrocellulose filters ("Synpor," Czechoslovakia) with a pore diameter of 0.6 μ. The filters were washed with 5% TCA, dried, placed under toluene scintillator, and their radioactivity was determined on a "Nuclear Chicago Mark II" liquid scintillation counter (USA). The level of radioactivity was calculated per milligram protein, per gram tissue, and per 100 g body weight. To determine the blood level of radioactivity 0.2 ml of whole blood was added to 10 ml of Bray's dioxan scintillator.

The protein content, concentration of cytochromes P-450 and b_5, and the aminopyrine N-demethylase and aniline p-hydroxylase activity were determined by methods described previously [4].

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Induction of the monooxygenase system by phenobarbital, against the background of deficiency of essential amino acids and vitamins, was expressed to a much greater degree than in animals on a balanced diet (Table 1). The content and activity of microsomal enzymes in the induced animals were independent of the character of the diet, with the exception of the cytochrome b_5 concentration, expressed per 100 g body weight, whereas in the control animals of group 2, receiving a diet deficient in essential components, the p-hydroxylase and N-demethylase activities,

TABLE 1. Effect of Multiple Nutrient Deficiency on Induction of Monooxygenase System of Liver Microsomes of Rats Receiving Three Injections of Phenobarbital in a Dose of 80 mg/kg (M ± m)

Parameters studied	Group 1 (balanced diet)		Group 2 (deficiency of lysine, methionine, threonine, and vitamins A, C, and E	
	control	treat. with PB	control	treatment with PB
p-Hydroxylase activity, nmoles reaction product/min/mg protein	0,84±0,03	1,38±0,04* (164,3)	0,54±0,02**	1,27±0,04* (235,2)
Per 100 g body weight	77,7±8,7	216,6±9,7* (278,8)	60,3±1,4	234,5±11,2* (388,9)
N-demethylase activity, nmoles reaction product/min/mg protein	4,18±0,17	11,02±0,36* (263,6)	3,47±0,10**	10,01±0,30* (288,5)
Per 100 g body weight	385,9±47,8	1727,9±51,5* (447,8)	392,9±32,6	1852,3±93,9* (471,4)
Content of cytochrome P-450, nmoles/mg protein	0,34±0,02	1,00±0,09* (294,1)	0,25±0,05	1,05±0,08* (420,0)
Per 100 g body weight	28,7±2,6	158,1±17,2* (550,9)	28,0±4,9	193,6±12,2* (691,4)
Content of cytochrome b_5, nmoles/mg protein	0,36±0,03	0,21±0,02* (58,3)	0,28±0,06	0,25±0,02 (89,3)
Per 100 g body weight	32,3±1,2	33,5±3,7 (103,7)	31,1±4,8	45,8±1,9 *,** (147,3)

Legend. Here and in Table 2, percentages of corresponding control shown between parentheses. *) Differences significant ($p < 0.05$) compared with corresponding control; **) differences significant ($p < 0.05$) compared with results obtained on animals of group 1, subjected to corresponding treatment.

TABLE 2. Effect of Multiple Nutrient Deficiency on Level of Radioactivity (cpm) of 2-^{14}C-Lysine into Fractions of Liver Homogenate, Femoral Muscle Homogenates, and Whole Blood of Rats Receiving Three Injections of Phenobarbital in a Dose of 80 mg/kg (M ± m)

Parameters studied	Group 1 (balanced diet)		Group 2 (deficiency of lysine, methionine, and vitamins A, C, and E)	
	control	treatment with PB	control	treatment with PB
		Liver microsomes		
Per milligram protein	1084±96	1015±82 (93,6)	868±122	1262±66* (145,4)
Per gram liver	30 157±3 414	38 148±3088 (126,5)	28 970±5 336	51 607±1509*,** (178,1)
Per 100 g body weight	97 950±8 246	158 601±10 964* (161,9)	100 846±13 842	233 311±13 424*,** (231,4)
		Cytosol		
Per milligram protein	908±106	895±85 (98,6)	800±118	1269±108*,** (158,6)
Per gram liver	93 658±10 386	88 777±8 394 (94,8)	80 612±11 284	134 252±14 235*,** (166,5)
Per 100 g body weight	308 827±39 473	369 528±31 173 (119,6)	278 146±28 945	610 341±78 981*,** (219,4)
		Residue after centrifugation of liver homogenates at 10,000 g		
Per milligram protein	734±83	792±139 (107,9)	581±72	864±36* (148,7)
Per gram liver	110 801±12 635	110 138±17852 (99,4)	95 446±13 546	126 256±5 899 (132,3)
Per 100 g body weight	365 864±49 413	456 784±69 896 (124,8)	334 920±31 387	575 864±61 678* (171,9)
		Homogenates of femoral muscle		
Per milligram protein	115±23	133±18 (115,6)	150±21	158±24 (105,3)
Per gram liver	28 414±5 047	32 130±4 721 (113,1)	35 581±5 926	37 798±5 308 (106,2)
		Blood		
In 0.2 ml whole blood	4662±498	4919±364 (105,5)	6642±727	7610±666** (114,6)

calculated per milligram protein, were lower than in the control animals of group 1, kept on a balanced diet. It was shown previously that this multiple nutrient deficiency leads to reduction of nearly all the parameters of monooxygenase function studied, whether expressed in relation to microsomal protein or per 100 g body weight in rats [3]. One result of this decrease in activity and content of enzymes for xenobiotic oxidation is a significant lengthening of the duration of phenobarbital sleep in the rats of group 2.

Attention must also be drawn to the fact that the difference in the degree of induction, depending on the nature of the diet, increased if activity and content of monooxygenases were expressed per 100 g body weight. The first explanation of this fact is that in the induced rats of group 2 the relative mass of the liver was increased by 34.8% compared with the control, whereas in animals on a balanced diet this parameter was increased by only 26.7% (Fig 1). We obtained similar results previously [2].

On the basis of these findings it can be concluded that the greater degree of induction of monooxygenases by PB against the background of multiple nutrient deficiency can be explained, on the one hand, by lowering of the "basal" level of function of this enzyme system and, on the other hand, by a greater increase in the relative weight of the liver.

As the results in Table 2 show, administration of PB to the rats of group 1 led to an increase of radioactivity by 61.9% compared with the control, only in the microsomal preparations when calculated per 100 g body weight. In all other cases this parameter did not differ from the control. After injection of PB into the animals of group 2, the radioactivity level rose significantly in all fractions of liver homogenate studied, when calculated by different methods, except radioactivity in the residue after centrifugation of the liver homogenate at 10,000*g* and calculated per gram of liver. In this case the degree of this increase rose significantly subsequently when calculated per gram of liver and per 100 g body weight. The level of radioactivity in microsomal preparations of induced rats of group 2, calculated per gram liver and per 100 g body weight, and by all methods of calculation in the cytosol, was higher by 35-65% than in the induced animals of group 1.

Levels of radioactivity in homogenates of the femoral muscle did not differ from each other depending on the character of the diet or administration of PB. Levels of radioactivity in whole blood of the induced animals of both groups likewise did not differ from the corresponding control values, although in animals receiving PB and on a diet with multiple nutrient deficiency, it was increased by a greater degree than in animals on a balanced diet. It must also be pointed out that the blood level of radioactivity in the induced animals of group 2 was 54.7% higher ($p < 0.02$) than in the induced animals of group 1 (Table 2).

It can be concluded that induction of microsomal enzymes under conditions of a diet deficient in certain essential components leads to mobilization of these factors in the liver from the other organs and tissues in order to maintain the detoxicating function of this organ. Although in muscle homogenates, in animals receiving PB and a diet with multiple deficiency, reduction of radioactivity was not observed, elevation of its level in whole blood of these animals above the blood level of radioactivity of the induced animals against the background of a balanced diet may be a reflection of the transport of the deficient essential lysine into the liver.

Further evidence that essential food components can flow to the liver from other organs and tissues in order to maintain the process of induction of microsomal enzymes is the fact that in the experimental animals of group 2, also exposed to vitamin A deficiency, after the second injection of PB blood-stained encrustations appeared around the eyes, evidence of the development of hypovitaminosis A, whereas no such development took place in the control animals of that group or in all animals of group 1. Similar phenomena have been described during induction by PB of the monooxygenase system of the liver in rats receiving a diet deficient in vitamin A [5]. Under these circumstances the content of cytochrome P-450 and activity of enzymes responsible for oxidizing xenobiotics reached the level observed in induced animals receiving an adequate supply of vitamin A. These workers also concluded that, if deficient in the diet, the vitamin A needed to induce microsomal monooxygenases is mobilized from other organs and tissues, and this aggravates the signs and symptoms of vitamin deficiency.

The results of this investigation thus confirm the previous hypothesis [3, 4] that induction of the microsomal monooxygenases of the mammalian liver depends strongly on the character of the diet. This dependence is evidently an important property of the animal and plays a major role in its adaptation to the surrounding medium.

LITERATURE CITED

1. A. I. Archakov, Microsomal Oxidation [in Russian], Moscow (1975).
2. T. Zh. Nurmagambetov, B. B. Amirov, T. K. Kuanysheva, et al., Vopr. Pitaniya, No. 1, 40 (1984).
3. T. Sh. Sharmanov, Vestn. Akad. Med. Nauk SSSR, No. 1, 28 (1985).
4. T. Sh. Sharmanov, T. Zh. Nurmagambetov, Sh. A. Bakanov, et al., Vopr. Med. Khim., No. 3, 106 (1985).
5. W. L. Backes, R. F. Krause, and W. J. Canady, Nutr. Rep. Int., **19**, 9 (1979).
6. T. Omura and R. Sato, J. Biol. Chem., **239**, 2370 (1964).