INDUCTION OF MONOOXYGENASE SYSTEM AND INCORPORATION OF RADIOACTIVE LABEL FROM 2-^{14}C-LYSINE INTO LIVER MICROSOMAL FRACTION OF PHENOBARBITAL-TREATED RATS FED A DIET DEFICIENT IN LYSINE, METHIONINE, THREONINE, AND VITAMINS A, C, AND E

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Induction of a cytochrome P-450-dependent monooxygenase system of mammalian liver microsomes by phenobarbital takes place through intensification of de novo protein synthesis [1]. Accordingly, and in line with the principles of sound nutrition, it has been correctly suggested that when the diet is deficient in essential components, the induction of this enzyme system, as an adaptive response to the action of xenobiotics, that are inducers of phenobarbital type, may lead not only to a redistribution of the flow of nutrients brought in with the diet, but also to the leakage of deficient essential components into the liver from other organs and tissues, which may adversely affect the other functions of the body and thereby aggravate the manifestations of nutritional polydeficiency.

To test this hypothesis experimentally we studied induction of the monooxygenase system and incorporation of radioactive label from 2-^{14}C-lysine into microsomes and certain other fractions of liver homogenate in phenobarbital-treated rats receiving a balanced diet or a diet deficient in three essential amino acids (lysine, methionine, and threonine) and vitamins A, C, and E.

EXPERIMENTAL METHOD

Experiments were carried out on 16 growing male WAG rats weighing initially 40-60 g, increasing to 160-200 g at the time of sacrifice. The animals, divided into two groups with eight rats in each group, were kept for the first 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 diet was given previously [4]. In each group the animals were divided into two subgroups: the experimental rats were given phenobarbital (PB; from "Merck," West Germany) for 3 days by intraperitoneal injection in a dose of 80 mg/kg, as a sterile solution in 0.9% NaCl; control rats received injections of the solvent only. During the 24 h before the first injection of PB, all the animals received intraperitoneal injections of 2-^{14}C-lysine ("Izotop," Leningrad), in a total dose of 80 μCi/100 g body weight, divided into four fractions, injected at intervals of 4 h. The quantity of radioactive lysine injected was 180 times less than the quantity needed to satisfy the daily requirement in the lysine-deficient 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 and the microsomal fraction was isolated [6]. To determine the level of radioactivity in the microsomal preparations, in the postmicrosomal supernatant, and in the cell residues after centrifugation of the liver homogenates at 10,000*g*, and in homogenates of the femoral muscle of the rats, aliquots of a suspension of the corresponding biomaterials containing 4 mg protein were added to 2 ml of 10% TCA and filtered through nitrocellulose filters ("Synpor," Czechoslovakia),

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TABLE 1. Effect of Nutritional Polydeficiency on Induction of Monooxygenase System of Liver Microsomes, after Three Injections of PB into Rats in a Dose of 80 mg/kg (M ± m)

Parameter studied	Group 1, control	Group 2		
		treated with PB	control	treated with PB
p-Hydroxylase activity, nmoles reaction product/min:				
per milligram 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				
per milligram 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:				
per milligram 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:				
per milligram 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: number in parentheses gives percentage of corresponding control; *$p < 0.05$ differences significant compared with corresponding control, **$p < 0.5$) compared with results obtained in animals of Group 1, subjected to the corresponding treatment.

with a pore diameter of 0.6 μm. The filters were washed with 5% TCA, dried, and covered with toluene scintillator, and radioactivity was determined on a liquid scintillation counter (Nuclear Chicago, Mark II, USA). The level of radioactivity was calculated per milligram protein, per gram tissue, and per 100 g body weight. To determine the level of radioactivity in the blood, 0.2 ml of whole blood was added to 10 ml of Bray's dioxan scintillator.

Parallel determinations of concentrations of protein and cytochromes P-450 and b_5, and of amidopyrine-N-demethylase and aniline-*p*-hydroxylase activity were undertaken in preparations of microsomes 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 PB in animals deficient in essential amino acids and vitamins was much more intensive than in animals with a balanced diet (Table 1). The content and activity of the microsomal enzymes in the induced animals were independent of the character of the diet, except the concentration of cytochrome b_5 calculated per 100 g body weight, whereas in the control animals of Group 2, on a diet deficient in essential components, the *p*-hydroxylase and N-demethylase activity expressed per milligram protein was significantly lower than in the control animals of Group 1, kept on a balanced diet. It was shown previously that this kind of nutritional polydeficiency leads to a significant decrease in nearly all parameters of monooxygenase function, calculated both relative to microsomal protein and per 100 g body weight of the rats [3]. The result of this decrease in activity and content of enzymes oxidizing xenobiotics is considerable lengthening of the duration of phenobarbital sleep in the rats of Group 2.

Attention had also to be paid to the fact that the difference in the degree of induction increased depending on the character of the diet when activity and content of monooxygenases were calculated per 100 g body weight. This can be explained, first, by the fact that in the induced rats of Group 2 the relative mass of the liver was 34.8% greater than in the control, whereas in animals receiving a balanced diet, the increase was only 26.7% (Fig. 1). We obtained similar results previously [2].

It can be concluded from these data that the high level of induction of monooxygenases by PB against a background of nutritional polydeficiency can be explained, on the one hand, by a lowered basal level of functional activity of this enzyme system and, on the other hand, by the greater degree of increase in the relative mass of the liver.

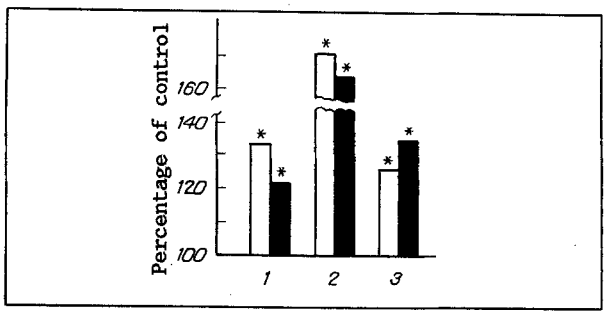

Fig. 1. Effect of three injections of PB in a dose of 80 mg/kg in rats on different diets, on content of microsomal protein and relative mass of liver in rats. Unshaded columns — balanced diet; black columns — diet deficient in lysine, methionine, threonine, and vitamins A, C, and E. 1) Content of microsomal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

It will be clear from the results in Table 2 that injection of PL into the rats of Group 1 caused an increase of 61.9% in radioactivity compared with the control in the microsomal preparations, calculated per 100 g body weight. In the remaining cases, this parameter did not differ from the control values. If PL was injected into the animals of Group 2, on a diet deficient in amino acids and vitamins, the level of radioactivity increased significantly in all fractions of liver homogenate studied, when calculated by different methods, with the exception of radioactivity in the residue after centrifugation of the liver homogenate at 10,000 g, calculated per gram of liver. In this case the degree of this increase rose successively, when calculated per gram of liver and per 100 g body weight. The level of radioactivity in microsomal preparations of the induced rats of Group 2, calculated per gram of liver and per 100 g body weight, and when calculated by all methods in the postmicrosomal supernatant, was significantly higher (by 35-65%) than in the induced animals of Group 1.

The level of radioactivity in femoral muscle homogenates did not differ significantly depending on the character of the diet or injection of PB (Table 2). The level of radioactivity in whole blood in the induced animals of both groups likewise did not differ from the corresponding control values, although when PB was injected into rats with nutritional polydeficiency, it was increased by a greater degree than in animals on a balanced diet. Incidentally, the level of radioactivity in the blood of 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 from these results that induction of microsomal enzymes under the conditions of an inadequate supply of certain essential dietary components leads to the 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 after treatment with PB, and against a background of nutritional polydeficiency, no reduction of radioactivity was observed, the excess of its level in the whole blood of these animals over its level in the blood of the induced animals receiving a balanced diet may be a reflection of transport of the deficient essential lysine into the liver.

Yet another piece of evidence in support of possible leakage of essential food components into 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 deficient in vitamin A, after the second injection of PB, blood-stained incrustations appeared around the eyes, evidence of the development of hypovitaminosis A, whereas in the control animals of this Group and all the animals of Group 1 this was not observed. A similar phenomenon was described by Backes and co-workers [5] during induction by PB of a monooxygenase system in the liver of rats on a diet deficient in vitamin A. In this case the content of cytochrome P-450 and activity of xenobiotic-oxidizing enzymes reached the level observed in the induced animals receiving an adequate amount of vitamin A. These investigators also concluded that vitamin A, essential for induction of microsomal monooxygenases, if deficient in the diet, is mobilized from other organs and tissues, leading to intensification of the symptoms and signs of vitamin deficiency.

Thus the results of this investigation confirm the previous hypothesis [3, 4] that induction of monooxygenases of mammalian liver microsomes depends to a considerable degree on the character of the diet. This dependence is evidently an important property of the body, playing an important role in its adaptation to the external medium.

TABLE 2. Effect of Nutritional Polydeficiency on Level of Radioactivity from 2-^{14}C-Lysine Incorporated in Fractions of Liver Homogenate, Homogenates of Femoral Muscle, and in Whole Blood of Rats Treated with PB in Three Doses Each of 80 mg/kg

Parameter studied	Group 1 (balanced diet)		Group 2 (diet deficient in lysine, methionine, threonine, and vitamins A, C, and E)	
	control	treatment with phenobarbital	control	treatment with phenobarbital
Liver microsomes				
per milligram protein	1084±96	1015±82 (93,6)	863±122	1262±66* (145,4)
per gram liver	30 157±3414	38 148±3088 (126,5)	28 970±5336	51 607±1509*,** (178,1)
per 100 g body weight	97 950±8246	158 601±10 964* (161,9)	100 846±13 842	233 311±13 424*,** (231,4)
Postmicrosomal supernatant of liver				
per milligram protein	908±106	895±85 (98,6)	800±118	1269±108*,** (158,6)
per gram liver	93 658±10 386	88 777±8394 (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 homogenate at 10,000g				
per milligram protein	734±83	792±139 (107,9)	581±72	864±36* (148,7)
per gram liver	110 801±12 635	110 138±17 852 (99,4)	95 446±13 546	126 256±5899 (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±5047	32 130±4721 (113,1)	35 581±5926	37 798±5308 (106,2)
Blood:				
in 0.2 ml whole blood	4662±498	4919±364 (105,5)	6642±727	7610±666** (114,6)

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