EFFECT OF PROTEIN ON INDUCTION AND RENEWAL OF THE CYTOCHROME P450 COMPLEX OF RAT LIVER

M. M. Gapparov, T. N. Zaboikina,
G. V. Nikol'skaya, and G. Yu. Mal'tsev

UDC 612.352.2-06:613.28

KEY WORDS: rat liver; protein; cytochrome complex.

The main parameters characterizing renewal of macromolecules are the velocity of synthesis and degradation, the stocks of precursors, and the rate at which newly synthesized molecules are transported to the place where they are utilized. Under the influence of factors such as hormones, food, drugs, and so on, after a temporary disturbance of equilibrium between synthesis and degradation of biopolymers the cell reaches a new steady state, characterized by new values of renewal velocity, i.e., a new steady flow of components in the biomembranes [6, 7].

This paper describes an investigation into the effect of different levels of protein in the diet on parameters of renewal of the protein components of the cytochrome P450 complex in rat liver microsomes, and also on the level of induction of this cytochrome by xenobiotics. The hypothesis was adopted that the rate of renewal of membrane components determines the volume and time of adaptation of the membrane systems to external influences and, in particular, to induction of cytochrome P450 after administration of xenobiotics.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing initially 200-300 g, kept for 35 days on diets with different protein contents (Table 1).

The rats received water and food *ad lib.*, and salt and vitamins on the scale given in [9].

Cytochrome P450 was induced in the rats by daily administration of phenobarbital in a dose of 100 mg/kg body weight for 5 and 10 days.

Cytochrome P450 was isolated by the method of stepwise fractionation with polyethylene glycol [2]. The content of cytochrome P450 in the turbid samples was measured [8] on a Hitachi-356 (Japan) spectrophotometer. Absolute spectra of a purified preparation of cytochrome P450 (oxidized, reduced, and reduced + CO) were recorded in phosphate buffer, pH 7.4, containing 20% glycerol at 20°C. A coefficient of molar extinction of -91 $mM^{-1} \cdot cm^{-1}$ was used in the calculations.

The DNA content in the homogenate was determined [4], taking the mean DNA content per liver cell to be $2.66 \cdot 10^{-6}$ µg [3], and the number of cells contained in 1 g liver was calculated.

The half-renewal time of the protein fraction of the cytochrome P450 complex was determined by the equation suggested by Arias [5]:

$$P_t = P_0 \cdot l^{-k_d \cdot t},$$

where P_t is the specific radioactivity of the protein at time t; P_0 the initial specific radioactivity of the protein; k_d the velocity constant of degradation; and t the time elapsing from injection of the isotope until sacrifice of the animal.

Laboratory of Isotopic Methods of Investigation, Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 43-46, April, 1982. Original article submitted May 28, 1981.

 0007-4888/82/9304-0434$07.50

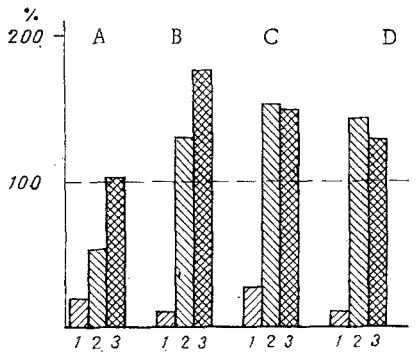

Fig. 1. Effect of protein deficient diet on content of cytochrome P450 complex, in mg protein/cell (A); on change in content of spectrophotometrically determined cytochrome P450, in μmoles/cell (B); on change in content of cytochrome P450 in isolated complex, in μmoles/mg protein of complex (C), and on total cytochrome P450 content in μmoles/g liver (D). Data shown in % of control diet (18% protein), taken as 100. 1) 4.5% protein; 2) 9%; 3) 36%.

TABLE 1. Ratio between Foodstuffs (in % of total calorific value of diet)

Protein, casein	Fat		Carbohydrates, starch
	lard	sunflower oil	
18 (control)	13	13	56
4,5	13	13	69,5
9	13	13	65
36	13	13	38

In semilogarithmic coordinates this relationship is described by a straight line; the angle of slope is defined by the value of k_d, which is related to the half-renewal time $T_{1/2}$ by the equation:

$$k_d = \frac{0.693}{T_{1/2}} [5].$$

The primary data were subjected to statistical analysis. To evaluate k_d, defining the slope of the straight line, the method of least squares was used.

D,L-[^{14}C]leucine was used as the radioactive precursor for protein synthesis. The labeled amino acid (50 μCi per rat) was injected intraperitoneally 1, 4, and 8 days before sacrifice. Protein in the sample was precipitated by TCA and the precipitate was washed with ether. Specific radioactivity of the proteins was determined by standard methods, using a PCS scintillator (Amersham, England) and the Rack-Betta 1215 LKB-Wallak scintillation counter, in cpm. The protein concentration was determined by the Lowry–Ciocalteu method [1].

EXPERIMENTAL RESULTS

Variations in the content of cytochrome P450 due to a diet unbalanced for protein are illustrated in Fig. 1. Protein deficiency in the diet led to a marked fall in the content of cytochrome P450 calculated per liver cell (Fig. 1B, Table 2), per gram liver (Fig. 1D, Table 2), and per milligram protein of the purified cytochrome P450 complex (Fig. 1C, Table 2). In animals kept on a 9% diet the total content of the cytochrome P450 complex, expressed as protein isolated from one cell (Fig. 1A, Table 2), fell whereas the content of spectrophotometrically determined cytochrome calculated per cell rose (Fig. 1B, Table 2), on account of an increase in the relative content of cytochrome P450 in 1 mg protein of the complex (Fig. 1C, Table 2). An excess of protein in the diet (36%) led to an increase in the cytochrome P450 content in the liver.

The graph in Fig. 2 can be used to determine the half-renewal time of the protein part of the cytochrome P450 complex (Table 2). A deficiency of protein in the diet led to a signifi-

TABLE 2. Effect of Diets Unbalanced for Protein on Isolated Cytochrome P450 Complex (M ± m, n = 4)

Protein content in diet, %	Quantity of cytochrome P450				Half-renewal time ($T_{1/2}$), days
	in mg/liter cell × 10^9	in μmoles/liver cell × 10^{13}	in μmoles/mg protein × 10^2	in μmoles/g liver × 10^3	
18 (control)	1,07±0,12	2,29±0,31	0,30±0,04	10,32±2,01	6,9
4,5	0,23±0,01	0,19±0,03	0,09±0,01	0,86±0,19	11,6
9	0,63±0,06	3,22±0,19	0,47±0,04	15,37±5,31	5,3
36	1,10±0,22	4,06±0,79	0,45±0,04	15,00±2,84	6,3

TABLE 3. Effect of Protein in Diet on Induction of Cytochrome P450 by Phenobarbital (M ± m, n = 3-4)

Protein content in diet, %	Induction of cytochrome P450	Administration for 5 days		Administration for 10 days	
		control	experiment	control	experiment
18	In mg protein/cell × 10^9	1,40±0,20	1,72±0,17	1,43±0,32	2,17±0,28
4,5		1,10±0,11	1,00±0,20	1,00±0,13	0,79±0,06
9		0,96±0,05	2,06±0,36	0,53±0,14	1,67±0,11
36		1,22±0,13	1,39±0,48	0,79±0,08	1,48±0,17
18	In μmoles P450/cell × 10^{13}	0,46±0,12	1,38±0,12	0,85±0,13	2,48±0,63
4,5		0,09±0,03	0,27±0,05	0,10±0,04	0,34±0,04
9		0,20±0,02	2,86±0,59	0,18±0,03	2,49±0,26
36		0,39±0,21	2,28±0,45	0,24±0,19	2,06±0,26
18	In μmoles P450/mg protein × 10^3	0,20±0,11	0,81±0,02	0,22±0,03	1,20±0,16
4,5		0,13±0,06	0,32±0,05	0,10±0,03	0,44±0,03
9		0,21±0,07	1,43±0,03	0,24±0,08	1,40±0,03
36		0,33±0,13	1,24±0,09	0,30±0,06	1,56±0,04
18	In μmoles P450/g liver × 10^3	16,9±5,0	92,1±9,3	12,5±1,1	120,0±45,1
4,5		6,6±1,7	28,2±1,3	5,8±1,4	27,2±5,2
9		8,2±2,0	142,0±12,1	9,7±0,2	196,3±19,6
36		24,1±6,0	129,6±29,4	17,6±3,1	145,8±16,8

cant increase in the half-renewal time from 6.9 days (control diet) to 11.6 days (low-protein diet). The half-renewal time of the cytochrome P450 complex in rats receiving 9 and 36% diets did not differ significantly from the control (Table 2).

A deficiency of protein in the diet thus led to a fall in the content of cytochrome P450 in the liver accompanied by a simultaneous increase in its half-renewal time, which was evidently necessary in order to preserve the detoxicating functions of the monoxygenase system of the microsomal membranes. Consequently, the results suggest that a protein deficiency in the diet lowers the rate of renewal of the protein moiety of the cytochrome P450 complex and of proteins structurally connected with it.

In another part of the investigation data were obtained on the effect of phenobarbital on the liver microsomes and their content of cytochrome P450. Two periods of administration of phenobarbital (5 and 10 days) were analyzed. Injection of phenobarbital over a period of 10 days, it will be noted gave the same pattern of results as the 5-day course, but the effect was more marked (Fig. 3, Table 3). Injection of phenobarbital caused induction of cytochrome P450 in the liver cells. This effect was seen particularly strongly in animals receiving diets containing 18 and 9% protein (Table 3). In rats receiving a 9% protein diet the cytochrome P450 content calculated per cell increased almost 14-fold, whereas the content of microsomal protein increased only twofold (Table 3; Fig. 3A, B). In rats receiving a low protein diet (4.5%) the content of cytochrome per cell increased threefold (Table 3, Fig. 3B), but the content of microsomal protein calculated per cell actually fell a little, i.e., the content of cytochrome P450 in the liver cells increased on account of an increase in its relative content in the microsomes (Fig. 3C). The greatest degree of induction of cytochrome P450 was thus observed in animals on a 9% protein diet, whereas in those on a 4.5% diet it was

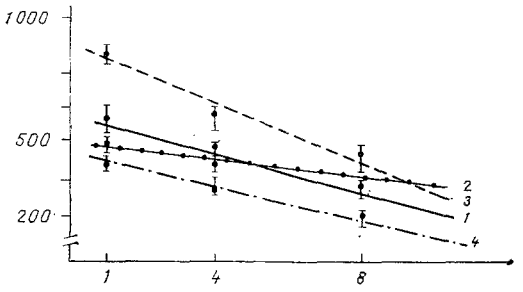

Fig. 2

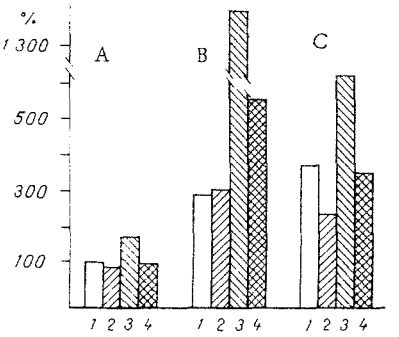

Fig. 3

Fig. 2. Kinetics of removal of [^{14}C]leucine from protein fraction of cytochrome P450 complex in animals on diets with different protein content. Abscissa, time from injection of isotope to sacrifice (in days); ordinate, radioactivity (cpm)/mg protein of cytochrome P450 complex. 1) 18%; 2) 4.5%; 3) 9%; 4) 36%.

Fig. 3. Induction of content of microsomal protein calculated per cell (A), of cytochrome P450 calculated per cell (B), and of cytochrome P450 calculated per milligram microsomal protein (C) by phenobarbital against the background of diets differing in their protein content. Value of parameter in unstimulated animals taken as 100%. 1) 18%, 2) 4.5%, 3) 9%, 4) 36% protein.

much lower, evidence of the importance of a parameter such as the rate of renewal for adaptation to xenobiotics.

Marked protein deficiency in the diet thus leads to a substantial fall in the cytochrome P450 content in the liver microsomes of rats accompanied by lengthening of the half-renewal time of the protein fraction of the cytochrome P450 complex. This lengthening of the half-renewal time was evidently aimed at preserving the detoxicating function of the liver cells, i.e., a quantitative deficiency of cytochrome P450 is compensated by lengthening of its half-renewal time. The role of slowing of cytochrome P450 metabolism is manifested in its induction by xenobiotics. With slower renewal of the protein moiety of the cytochrome P450 complex in animals kept on a low protein (4.5%) diet, the inducer gave a smaller quantitative effect of stimulation of the cytochrome P450 content compared with this parameter in rats receiving a diet with 9 and 36% protein. Accordingly, when the mechanism of action and the inducing doses of xenobiotics are studied, the composition of the diet received by the experimental animals (especially its content of first-class protein) must be taken into account.

LITERATURE CITED

1. J. Bailey, Techniques in Protein Chemistry, 2nd edn., Elsevier (1967).
2. I. I. Karuzina, G. I. Bachmanova, D. E. Mengazetdinov, et al., Biokhimiya, 44, 1049 (1977).
3. A. Lehninger, Biochemistry, Worth (1970).
4. M. G. Trudolyubova, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), pp. 313-316.
5. J. M. Arias, D. Doyle, and R. T. Schimke, J. Biol. Chem., 244, 3303 (1969).
6. F. W. Booth and J. O. Hollosry, Arch. Biochem., 167, 674 (1975).
7. D. J. Millward, Proc. Nutr. Soc., 38, 77 (1979).
8. T. Omura and R. Sato, J. Biol. Chem., 239, 2379 (1964).
9. P. D. Saville, J. Nutr., 91, 353 (1967).