though by a lesser degree. Complete cessation of the inhibitory effect of oligomycin on ATPase activity was observed when the Ba^{++} and Zn^{++} concentrations in the incubation medium were increased to 0.5 mM.

Such marked selective sensitivity of NaCl-stimulated ATPase activity to Ca^{++} and Mg^{++} ions could indicate that these cations are regulators of the activity of this enzyme. In fact, the data given in Table 2 shows that in control animals, in six of nine cases in membranes isolated in medium without EDTA, ATPase activity sensitive to NaCl and to oligomycin cannot be detected at all, even when EDTA is present in the incubation medium, whereas isolation of membranes in medium with EDTA facilitates the appearance of ATPase activity sensitive to oligomycin. It should, however, be pointed out that in this case also NaCl had hardly any effect on the velocity of ATP hydrolysis. Meanwhile, in animals receiving histamine, in all cases NaCl-stimulated, oligomycin-inhibited ATPase activity appeared both in membranes isolated in medium without EDTA and in those isolated in the presence of this substance.

The results, however, do not give an answer to the question of what order of events leads to activation of NaCl-stimulated ATPase by histamine. It may be that histamine lowers the affinity of this enzyme for bivalent cations, and this is accompanied by conformational changes in the protein and the transition of NaCl-stimulated ATPase into the active state as a result of the outflow of cations from their binding sites. The possibility likewise cannot be ruled out that histamine changes membrane permeability and thereby regulates interaction of this enzyme with bivalent cations.

LITERATURE CITED

1. V. S. Shubin, I. A. Duvakin, and V. A. Yurkiv, Biokhimiya, No. 6, 990 (1981).
2. B. S. Hill and D. E. Hanke, J. Membr. Biol., 51, 185 (1979).
3. M. H. Humphreys and L. Y. N. Chou, Am. J. Physiol., 236, E70 (1979).
4. Y. F. Lai and J. S. Thompson, Biochim. Biophys. Acta, 233, 84 (1971).
5. M. Morisawa and S. Utida, Biochim. Biophys. Acta, 445, 458 (1976).
6. W. B. Rathbun and M. B. Betlach, Anal. Biochem., 28, 436 (1969).

EFFECT OF PROTEIN INTAKE ON PROTEIN TURNOVER IN SUBCELLULAR FRACTION OF RAT LIVER

G. Yu. Mal'tsev, G. V. Nikol'skaya, and M. M. Gapparov

UDC 612.352.3-06:612.398

KEY WORDS: protein turnover; subcellular fractions; low- and high-protein diets.

Protein turnover in intracellular structures is a process which controls the steady-state content of the various proteins which provide for a definite level of functional activity. The important role of turnover in adaptive changes in response to the action of an external factor will thus be evident. The dietary factor is particularly important, for besides the function of participating in metabolism, it also performs the role of supplier of precursors for synthesis of biological macromolecules and, in particular, amino acids.

Theoretical analyses and numerous experiments [4, 7, 8] have broadened opportunities for the study of rates of protein renewal with the use of labeled amino acids. However, they have so far found only limited application in the field of biochemistry of nutrition.

The aim of this investigation was to assess dependence of protein turnover in subcellular fractions on long-term feeding on low- and high-protein diets.

Laboratory of Isotope 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 S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 8, pp. 69-72, August, 1982. Original article submitted February 12, 1982.

0007-4888/82/9408-1092$07.50

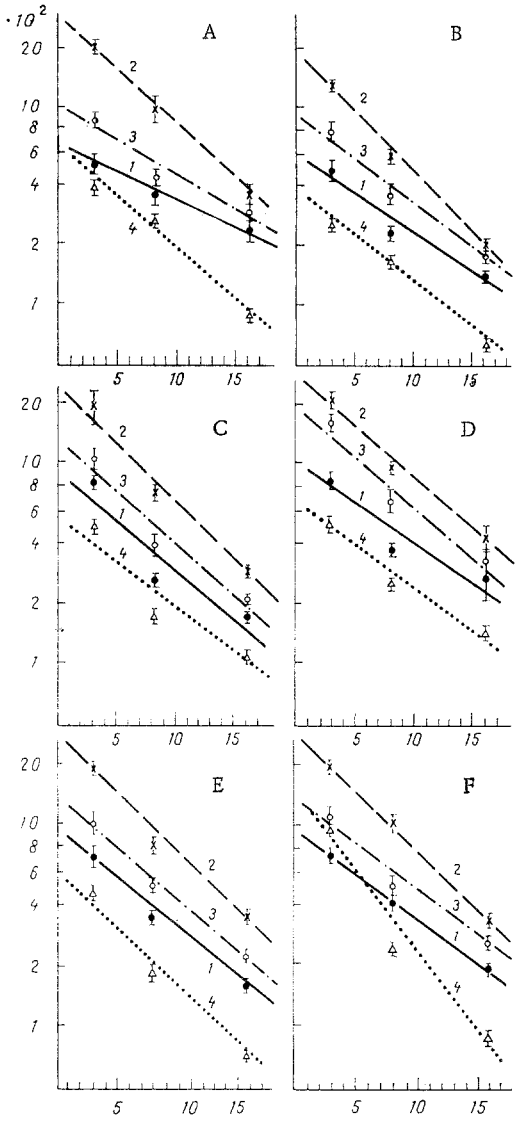

Fig. 1. Kinetics of elimination of [^{14}C]lysine from proteins of subcellular fractions of liver from rats receiving diets differing in protein content: 1) 18%, 2) 4.5%, 3) 9%, 4) 36% protein. A) Mitochondria; B) inner membranes of mitochondria; C) outer membranes of mitochondria; D) microsomes (after purification); E) lysosomes; F) cytosol. Abscissa, time after injection of isotope (in days); ordinate, specific radioactivity of fractions (in cpm/mg protein). At each point of time there were four or five animals.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 250-300 g were kept for 36 days on four different diets: control — 18% protein, two low-protein diets — 4.5 and 9% protein, and a high-protein diet — 36% protein by calorific value. The protein used was milk casein. All animals received 20% of a mixture of bacon + sunflower oil, 4% of a salt mixture, vitamin mixture, and fat-soluble vitamins. The rest of the diet was made up of carbohydrates (starch).

Subcellular fractions were isolated from rat liver homogenates [3]. The animals were anesthetized with ether, the liver removed and washed in cold physiological saline, after which it was forced through a metal press with a pore diameter of 0.8 mm. Homogenization was carried out in medium containing 0.25 M sucrose and 5 mM Tris-HCl, pH 7.4, in a Potter—Elvehjem homogenizer in the ratio of 1:4 (w/v). The isolated subcellular fractions were washed again, diluted in a minimal volume of medium, and kept at between −10 and −15°C. The microsomal frac-

TABLE 1. Some Characteristics of Protein Turnover in Subcellar Fractions of Rat Liver with Variation in Protein Content of Diet (M ± m)

Subcellular fraction	Protein content of diet, %	$t_{1/2}$, days	k_D, day^{-1}	Protein (P), mg/g liver	$k_D \cdot P$, mg/day
Mitochondria	18	11,6	0,06	52,1±4,0	3,13
	4,5	5,3	0,13	39,0±5,6	5,07
	9	8,66	0,08	47,5±5,0	3,8
	36	5,8	0,12	52,7±6,8	6,32
Inner mitochondrial membranes	18	7,7	0,09	32,3±3,0	2,91
	4,5	4,95	0,14	25,7±2,6	3,6
	9	6,93	0,10	27,7±6,3	2,77
	36	6,3	0,11	30,3±0,4	3,33
Outer mitochondrial membranes	18	6,3	0,11	18,8±2,9	2,07
	4,5	4,95	0,14	14,5±2,7	2,03
	9	5,78	0,12	14,5±5,0	1,74
	36	6,3	0,11	21,1±3,4	2,32
Microsomes	18	7,7	0,09	29,3±4,4	2,64
	4,5	5,8	0,12	19,1±2,1	2,3
	9	6,2	0,11	21,4±2,0	2,35
	36	6,93	0,1	30,6±7,1	3,06
Lysosomes	18	6,3	0,11	56,7±11,0	6,24
	4,5	5,3	0,13	37,1±9,1	4,82
	9	6,3	0,11	35,1±1,1	3,85
	36	5,3	0,13	56,0±6,1	7,28
Cytosol	18	6,93	0,1	277±22	27,7
	4,5	4,95	0,14	142±12	19,9
	9	6,3	0,11	202±70	22,2
	36	3,85	0,18	269±80	48,4

tion was purified [1] and the mitochondrial fraction separated by osmotic shock [6] into a fraction of external and a fraction of internal membranes.

Protein turnover in the subcellular fractions was estimated from the kinetics of outflow of labeled amino acid from the proteins. For this purpose, the animals were given an intraperitoneal injection of D,L-[^{14}C]lysine in a dose of 50 μCi per animal 3, 8, and 16 days before sacrifice. The half-elimination time was determined graphically by means of straight lines plotted between semilogarithmic coordinates of specific radioactivity versus time after injection of the isotope.

Radioactivity in the proteins was determined after their precipitation with 10% cold TCA and washing the residue twice with ether. After the residue had been dissolved in 0.02 N NaOH some of the solution was taken for protein determination [5], the rest for measurement of radioactivity in PCS scintillation medium (Amersham, England) on a Rack-Beta counter (Sweden–Finland), by means of calibration curves.

EXPERIMENTAL RESULTS

The time course of specific radioactivity of proteins of the subcellular fractions is shown in Fig. 1. The straight lines drawn between coordinates of log of specific radioactivity and time after injection of [^{14}C]lysine were plotted on the basis of regression coefficients calculated by the method of least squares. Values of the half-turnover time ($t_{1/2}$), found from the straight lines, and the corresponding values of the velocity constant of degradation (k_D) on the basis of the equation $k_D = \ln 2t_{1/2}$, are given in Table 1. A characteristic factor for all the subcellular fractions studied was a marked decrease in $t_{1/2}$ in the group of animals receiving a diet containing 4.5% protein compared with this parameter in the control animals (diet with 18% protein). In animals receiving a diet with 9% protein, a very small decrease in $t_{1/2}$ was observed for certain fractions. Meanwhile, in the group of animals receiving a high-protein diet (36%), a marked decrease in $t_{1/2}$ also was observed, especially in the mitochondrial fraction, the inner membranes of the mitochondria, and the cytosol. Thus, in animals receiving both high- and low-protein (especially 4.5%) diets an increase in turnover of total proteins of the fractions was observed, especially mitochondrial proteins (inner membranes) and soluble cytoplasmic proteins. A similar fact was discovered by other workers [2] who observed a decrease in $t_{1/2}$ in subcellular fractions from animals receiving a diet with 3.4% protein for 2 weeks.

The results in Table 1 show that the protein content was reduced in all fractions: by a greater degree in rats receiving a diet with 4.5% protein, by a lesser degree in rats receiving a diet with 9% protein. The protein content in all fractions was unchanged for rats kept on a diet with 36% protein.

The product $k_D \cdot P$, where P is the protein content in the fraction (in mg) characterizes the rate of protein degradation (under steady-state conditions, the rate of protein turnover). As Table 1 shows, the rate of protein turnover in rats receiving a high-protein diet was higher in all fractions than in the control, chiefly on account of an increase in the velocity constant of degradation (for steady-state conditions — the velocity constant of turnover). As regards the groups of animals receiving a low-protein diet, their turnover rates were reduced in most fractions except mitochondrial, on account of a decrease in the protein content (P), by a greater degree despite an increase in the value of k_D.

The value of the turnover rate $k_D \cdot P$ or the flow of replaceable protein molecules in fractions except mitochondrial thus depends directly on the protein content in the diet, i.e., with an increase in the protein content in the diet the rate of protein turnover also increases, and this is particularly characteristic of the fraction of soluble cytosol proteins. Despite this rule, the fraction of replaceable proteins relative to the total reserves of proteins was increased in rats receiving diets containing both 4.5 and 36% protein, possibly due to an increase in the fraction of short-living proteins. It is evidently this which determines the character of adaptive changes in the cell providing for its physiological activity. Cytochrome oxidase activity was measured as a parameter of cell function. Specific activity in the fraction of inner membranes for animals receiving diets containing 4.5, 9, 18, and 36% protein amounted to 0.8, 0.35, 0.44, and 0.34 μmole substrate/min/mg protein, respectively, and total activity was 20.5, 9.7, 14.1, and 10.3 μmoles substrate/min per fraction. The observed increases in total specific cytochrome oxidase activity in rats kept on a low-protein (4.5%) diet probably reflect induction of cytochromes, which leads to intensification of turnover of mitochondrial proteins on account of the shorter life span of the cytochromes. Meanwhile, intensification of protein turnover in animals receiving a high-protein diet was not due to cytochromes, but to increased synthesis of other short-living mitochondrial proteins.

LITERATURE CITED

1. J. DePierre and G. Dallner, in: Biochemical Investigation of Membranes [Russian translation], Moscow (1979), pp. 75-121.
2. P. R. Dallman and E. C. Manies, J. Nutr., 103, 257 (1973).
3. C. De Duve, B. C. Pressman, R. Gianetti, et al., Biochem. J., 60, 604 (1955).
4. R. D. Glass and D. Doyle, J. Biol. Chem., 247, 5234 (1972).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
6. D. S. Parsons and G. P. Williams, Methods Enzymol., 10, 443 (1967).
7. R. T. Schimke, Mol. Pharmacol., 1, 149 (1965).
8. R. Zak, A. F. Martin, and R. Blough, Physiol. Rev., 59, 407 (1979).