

Cell-free Translation of Carbamyl Phosphate Synthetase I and Ornithine Transcarbamylase Messenger RNAs of Rat Liver

EFFECT OF DIETARY PROTEIN AND FASTING ON TRANSLATABLE mRNA LEVELS*

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Total RNA of rat liver was translated in a cell-free system derived from rabbit reticulocytes, and synthesized precursor forms of carbamyl phosphate synthetase I (EC 6.3.4.16) and ornithine transcarbamylase (EC 2.1.3.2) were isolated by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Synthesis of both enzyme precursors was optimal with 90 to 180 mM potassium ion, 1.2 mM magnesium, and 50 μ g/ml of rat liver tRNA. Enzyme synthesis increased with total RNA up to 1.2 mg/ml and with time up to 90 min at 25 °C. Translatable levels of hepatic mRNAs for carbamyl phosphate synthetase I and ornithine transcarbamylase were 4.2- and 2.2-fold higher, respectively, in the case of rats fed a 60% casein diet as compared with rats fed a 5% casein diet. The differences in mRNA levels were slightly higher than the differences in the levels of the enzyme activities (3.3- and 1.9-fold, respectively) and of the enzyme proteins (3.3- and 2.1-fold, respectively). The results indicate that the dietary protein-dependent changes in the levels of carbamyl phosphate synthetase I and ornithine transcarbamylase are due mainly to changes in levels of translatable mRNAs for the enzymes. The hepatic levels of the two enzymes expressed per g wet weight of liver were also increased 1.7- and 1.3-fold, respectively, during a 7-day fasting over those of control animals kept on a 24% protein diet. However, the levels of translatable mRNAs for carbamyl phosphate synthetase I and ornithine transcarbamylase were decreased to 54 and 67%, respectively, of those of the control animals. Thus, the increase of the two enzyme levels during fasting appears to be the result of a decreased rate of degradation of the enzymes.

Carbamyl phosphate synthetase I (EC 6.3.4.16) and ornithine transcarbamylase (EC 2.1.3.2) catalyze the first two steps of urea synthesis in the liver of ureotelic animals. The former enzyme consists of two identical subunits of $M_r = 156,000$ – $160,000$ (1–4), is located in the mitochondrial matrix (5), and constitutes 20–30% of total matrix protein (2, 6). Ornithine transcarbamylase is a trimer of identical subunits

of $M_r = 35,300$ – $39,600$ (7–10) and is also located in the mitochondrial matrix (5, 9).

Rats subjected to different levels of protein intake and to fasting show significant changes in levels of enzyme proteins and activities of these two enzymes (11, 12). Cell-free synthesis of precursor forms of carbamyl phosphate synthetase I (pCPS) (13, 14) and ornithine transcarbamylase (pOTC) (15, 16) have recently been reported. We have extended our studies (14, 16) to investigate the mechanism(s) underlying the diet-dependent changes in the levels of these mitochondrial enzymes.

In this paper, we report optimal conditions for cell-free translation and demonstrate that the dietary protein-dependent changes in the levels of carbamyl phosphate synthetase I and ornithine transcarbamylase are due mainly to altered levels of functional mRNAs for the enzymes. In contrast, during fasting, the increase in levels of these enzymes is associated with a decrease in levels of translatable mRNAs for these enzymes.

EXPERIMENTAL PROCEDURES

Purification of Enzymes—Rat liver carbamyl phosphate synthetase I (14) and bovine liver ornithine transcarbamylase (7) were purified to homogeneity as described previously. Rat liver ornithine transcarbamylase was purified to near homogeneity as described previously (16).

Preparation of Antibodies—Antibodies against rat carbamyl phosphate synthetase I (14) and bovine ornithine transcarbamylase (16) were raised in rabbits and purified by three successive $(\text{NH}_4)_2\text{SO}_4$ precipitation at 40% saturation followed by affinity chromatography on enzyme-bound Sepharose as described previously. Immunoglobulin G was determined using an extinction coefficient of $E_{280\text{ nm}}^{1\%} = 13.5$.

Isolation of RNA—Male Wistar rats weighing 150–200 g were fed a diet containing 60% casein *ad libitum* for 8 days, unless otherwise indicated. Total hepatic RNA was isolated by phenol/NaDodSO₄ extraction as described previously (14). RNA was estimated using an extinction coefficient of $E_{260\text{ nm}}^{1\%} = 200$.

Cell-free Protein Synthesis—The assay mixture (50 μ l, pH 7.6) contained 28 mM Hepes,¹ 120 mM potassium acetate, 1.2 mM magnesium acetate, 1.1 mM ATP, 28 μ M GTP, 9 mM creatine phosphate, 15 μ g of creatine phosphokinase (Sigma), 2.2 mM dithiothreitol, 28 μ M each of an amino acid mixture minus methionine, about 10 μ Ci of [³⁵S]methionine (400 to 600 Ci/mmol; New England Nuclear), 15 μ l of nuclease-treated rabbit reticulocyte lysate (17), 2 μ g of rat liver tRNA, 0.15 mM each of protease inhibitors (antipain, leupeptin, chymostatin, and pepstatin), and various amounts of total hepatic RNA. Incubation was carried out at 25 °C for 90 min. To assay total protein synthesis, 2- μ l aliquots of the reaction mixture were spotted on Whatman 3MM paper strips and boiled for 10 min in 10% trichloroacetic acid. The paper strips were washed successively with water, ethanol, and ace-

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¹ The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; pCPS, a precursor for carbamyl phosphate synthetase I; pOTC, a precursor for ornithine transcarbamylase; NaDodSO₄, sodium dodecyl sulfate.

tone, and then dried and counted for radioactivity in a toluene scintillant (4.0 g of diphenyloxazole/liter).

Immunoprecipitation—After cell-free translation, the mixture was treated with 1.0 ml of a solution containing 10 mM Tris-HCl, 0.1% NaDodSO₄, 0.1% Triton X-100, 2 mM EDTA, the four protease inhibitors (0.2 mM each) (pH 7.4), and 13 μ g of affinity-purified anti-rat carbamyl phosphate synthetase I or anti-bovine ornithine transcarbamylase and incubated for 60 min at 25 °C. Protein A-bearing *Staphylococcus aureus* cells (50 μ l of 10% suspension), prewashed with the buffer used above containing no protease inhibitors, were then added, and the mixture was incubated for 60 min at 25 °C with shaking at 100 oscillations/min. The cells were washed three times with the same buffer (1 ml) and extraction was performed as described by Maccacchini *et al.* (18). NaDodSO₄-polyacrylamide gel electrophoresis was performed by the method of Laemmli (19) and the band of pCPS or pOTC was visualized by fluorography (20). The diphenyloxazole-impregnated and dried gels were cut into 2-mm strips and counted in a toluene scintillant with an efficiency of about 77%.

Enzyme Assays—Livers (about 0.5 g) were homogenized in 10 volumes of 20 mM potassium Hepes, 1% Triton X-100, 20% (w/v) glycerol, 1 mM dithiothreitol (pH 7.4), and the 100,000 \times *g* supernatant was measured for the enzyme activities. Carbamyl phosphate synthetase I was assayed isotopically (21) and ornithine transcarbamylase was assayed colorimetrically (7).

Other Methods—Protein diets were prepared as described by Harper (22). *S. aureus* cells (a Cowan I strain) were cultured as described by Kessler (23) in 3.7% Bacto-Brain heart infusion (Difco). Rat liver carbamyl phosphate synthetase I and ornithine transcarbamylase were labeled with [¹⁴C]formaldehyde (10 mCi/mmol; New England Nuclear) by the method of Rice and Means (24). Radial immunodiffusion was performed on glass plates (5 \times 10 cm) in 1% agarose containing 20 mM potassium phosphate (pH 7.4), 0.15 M NaCl, 3 mM sodium azide, and antibody. After diffusion for 4 days at room temperature, the agar was washed with 0.15 M NaCl, dried, stained with Coomassie blue, and destained (25, 26).

Materials—Nuclease from *S. aureus* was obtained from Boehringer Mannheim GmbH and microbial protease inhibitors (antipain, leupeptin, chymostatin, and pepstatin) were from Peptide Institute, Osaka, Japan. Rat liver tRNA, prepared by a phenol extraction method (27), was provided by Dr. K. Igarashi (Chiba University).

RESULTS AND DISCUSSION

Optimization of Immunoprecipitation—Previous studies (14, 16) showed that indirect immunoprecipitation of precursors of carbamyl phosphate synthetase I and ornithine synthetase using highly purified antibodies and fixed *S. aureus* cells gave better results than a direct method or an indirect method using a second antibody. Maximal precipitation of newly synthesized pCPS was obtained with 6 μ g of affinity-purified anti-carbamyl phosphate synthetase I and 50 μ l of 10% (w/v) *S. aureus* cells (Fig. 1). Similar results were obtained with pOTC and anti-bovine liver ornithine transcarbamylase (data not shown). Immunoprecipitation in the presence of 0.1% NaDodSO₄ plus 0.1% Triton X-100 gave lower background radioactivity than in the presence of 1% Triton X-100 alone without any significant loss of radioactivity in pCPS or pOTC.

Optimization of Reticulocyte Cell-free Translation System for the Synthesis of pCPS and pOTC—The time course of pCPS and pOTC synthesis in the rabbit reticulocyte lysate system was determined in order to optimize incubation conditions (Fig. 2A). Total protein synthesis was linear for about 30 min and continued at a reduced rate up to 90 min. Synthesis of full-length pCPS was first detected after about 30 min and increased up to 90 min, whereas pOTC synthesis was detectable in about 10 min and increased up to 90 min. The difference in lag time is consistent with the relative molecular weights of pCPS and pOTC (165,000 and 39,000, respectively).

Total protein synthesis increased with increasing concentration of total hepatic RNA up to and beyond 1.2 mg/ml (Fig. 2B). Synthesis of pCPS and pOTC increased with the

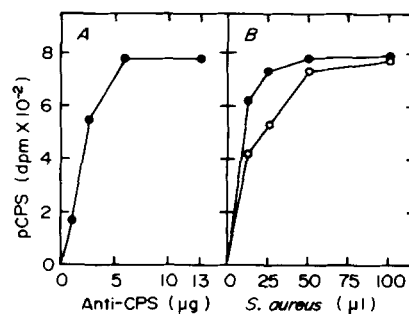


FIG. 1. Effect of various amounts of anti-carbamyl phosphate synthetase I (A) and fixed *S. aureus* cells (B) on immunoprecipitation of pCPS synthesized *in vitro*. Cell-free translation was performed using 1.2 mg/ml of total hepatic RNA. The translation mixture was then treated with 1.0 ml of 10 mM Tris-HCl, 0.1% NaDodSO₄, 0.1% Triton X-100, 2 mM EDTA, and protease inhibitors (0.2 mM each) (pH 7.4), and immunoprecipitation was performed as described under "Experimental Procedures" with (A) various amounts of affinity-purified anti-carbamyl phosphate synthetase I and 50 μ l of 10% *S. aureus* cells, or (B) 13 μ g of anti-carbamyl phosphate synthetase I and various amounts of *S. aureus* cells of two different batches (●, ○).

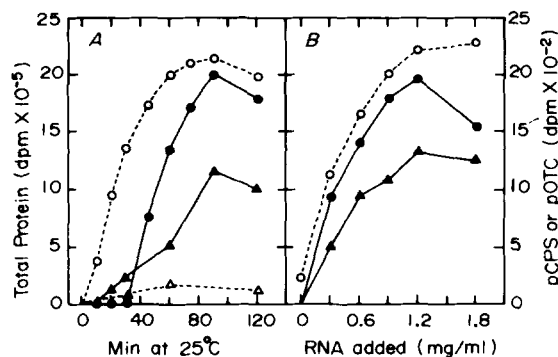


FIG. 2. Cell-free synthesis of total protein, pCPS, and pOTC as a function of time (A) and exogenous RNA concentration (B). Cell-free translation was performed (A) with 1.2 mg/ml of total hepatic RNA for various times, or (B) with various concentrations of exogenous RNA for 90 min. Synthesis of total protein was done with (○) or without (△) added RNA; pCPS (●) and pOTC (▲) with added RNA was measured as described under "Experimental Procedures."

addition of up to 1.2 mg/ml of total RNA and then declined.

The optimal concentration of potassium ion required for the synthesis of total protein, pCPS, and pOTC was between 90 to 180 mM when potassium acetate was used (Fig. 3A). The requirement for potassium ion was almost absolute for the synthesis of total protein and pOTC, whereas some pCPS was synthesized without added potassium ion. Substitution of potassium chloride for potassium acetate gave a sharp optimum at 80 mM, probably as a result of the inhibition caused by higher concentrations of chloride (28). The optimal concentration of magnesium for the synthesis of total proteins, pCPS, and pOTC was 1.2 mM when 1.1 mM ATP was included (Fig. 3B). Spermidine at concentrations of 0.05 to 0.4 mM did not significantly stimulate the synthesis of total protein, pCPS, and pOTC when 1.2 mM magnesium was used. When magnesium concentration was reduced to 0.8 mM, spermidine (0.6 mM) stimulated the synthesis of total protein and pCPS by 1.5- and 2.2-fold, respectively. Rat liver tRNA at a concentration of 50 μ g/ml stimulated the synthesis of total protein and pCPS by 1.4- and 2.2-fold, respectively (Fig. 3C).

Effects of Dietary Protein on Levels of Translatable mRNA for the Enzymes—The content of carbamyl phosphate syn-

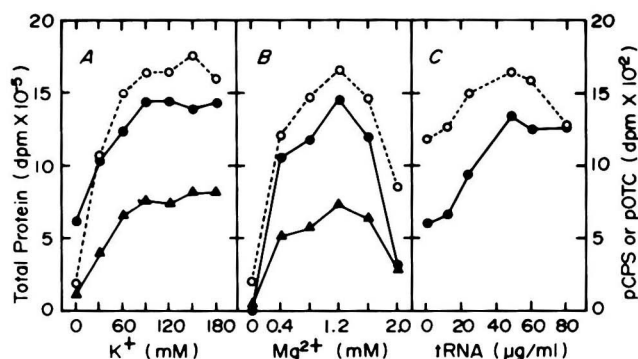


FIG. 3. Effect of potassium (A), magnesium (B), and tRNA concentrations (C) on the synthesis of total protein, pCPS, and pOTC. Cell-free translation was performed with 1.2 mg/ml of total hepatic RNA with various concentrations of potassium acetate (A), magnesium acetate (B), and rat liver tRNA (C). Except for the indicated variations, standard conditions were used. Synthesis of total protein (○), pCPS (●), and pOTC (▲) was measured as described under "Experimental Procedures."

thetase I and ornithine transcarbamylase in rat liver varies severalfold depending on dietary protein intake (11). In order to investigate the basis for this, functional mRNA levels for the enzymes were measured by means of cell-free translation. Total hepatic RNA was isolated from rats fed on a 5 or 60% casein diet for 8 days and then translated in a reticulocyte lysate system under optimized conditions. When total product was analyzed by NaDodSO₄ gel electrophoresis and fluorography, a faint band was observed at the position of pCPS (Fig. 4, lanes 2 and 3). Total RNA from a rat fed on a high protein diet was apparently more active than that from a rat fed on a low protein diet in the synthesis of this polypeptide. The band of pOTC could not be identified because of many radioactive polypeptides near the expected position. Immunoprecipitation of the reaction mixture with highly purified antibodies to rat liver carbamyl phosphate synthetase I and bovine liver ornithine transcarbamylase and analysis of the immunoprecipitates on a NaDodSO₄ gel gave the results shown in Fig. 4 (lanes 4 to 9). The synthesis of full-length pCPS and pOTC increased with increasing amounts of RNA prepared from both groups of rats. To be noted is that RNA isolated from a rat fed on a 60% casein diet was more active than that from a rat fed on a 5% casein diet in the synthesis of both pCPS and pOTC. The radioactive polypeptides moving faster than pCPS are presumably the result of incomplete synthesis of pCPS or its proteolysis during the translation and immunoprecipitation, because these peptides were seen only when anti-carbamyl phosphate synthetase was used and because radioactivity in these peptides closely paralleled that in full-length pCPS.

Incorporation of radioactivity into total protein, full-length pCPS, and pOTC is shown in Fig. 5. Hepatic RNA from rats fed a high protein diet was 1.2-fold more active than that from rats fed on a low protein diet in total protein synthesis. On the other hand, increase of pCPS synthesis by a high protein diet was greater, by 4- to 5-fold (mean, 4.2-fold), at every concentration of added RNA. A high protein diet resulted in an increase of about 2.2-fold in pOTC synthesis.

Table I summarizes experiments in which the enzyme levels are compared with the *in vitro* translational activity of total RNA. Rats fed a 60% casein diet had higher levels of carbamyl phosphate synthetase I and ornithine transcarbamylase activities by 3.3- and 1.9-fold, respectively, than rats fed a 5% casein diet.

Determination of the enzyme proteins by radial immunodiffusion confirmed the previous results (11) that dietary protein-dependent changes in the enzyme activities are due to

changes in concentrations of the enzyme proteins. The level of translatable mRNA for carbamyl phosphate synthetase I, expressed as a percentage of pCPS synthesis in total protein synthesis, was 3.3-fold higher in high protein diet-fed rats than in low protein diet-fed animals; the level of translatable mRNA for ornithine transcarbamylase was 1.8-fold higher.

Incubations with mixtures of equal amounts of hepatic RNA preparations from animals kept on low and high protein diets gave values for synthesis of pCPS and pOTC which approximated the mean for the values obtained from separate incubations with each RNA preparation (Table II). Thus, it is unlikely that the difference observed in translatable levels of mRNA from rats on low and high protein diets is due to the presence of an activator or inhibitor in one of the hepatic RNA preparations.

Nicoletti *et al.* (29) have reported that the half-life of carbamyl phosphate synthetase I in rats fed a 75% casein diet

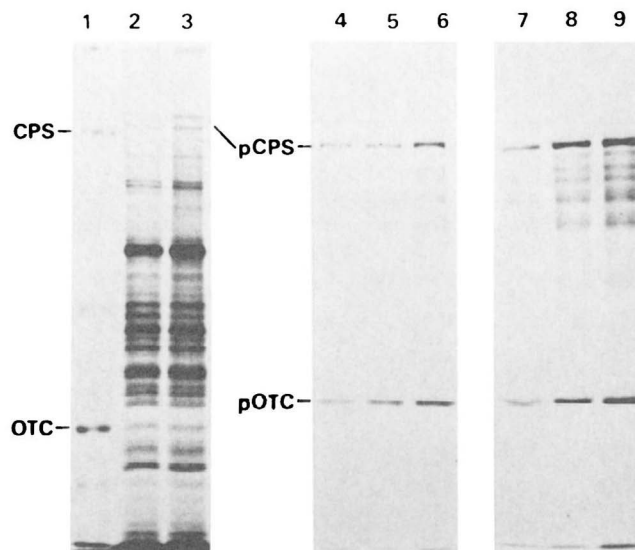


FIG. 4. Total product, pCPS, and pOTC synthesized *in vitro* with total hepatic RNA isolated from rats fed on different protein diets. Cell-free translation was performed with 0.4 mg/ml (lanes 4 and 7), 0.8 2, 3, 5, and 8), or 1.2 mg/ml (lanes 6 and 9) of total hepatic RNA from a rat fed on a 5% (lanes 2 and 4 to 6) or 60% (lanes 3 and 7 to 9) casein diet. Total product (lanes 2 and 3) and pCPS plus pOTC (lanes 4 to 9) were analyzed on NaDodSO₄-10% polyacrylamide gels which were then fluorographed. Lane 1, carbamyl phosphate synthetase I (CPS) and ornithine transcarbamylase (OTC).

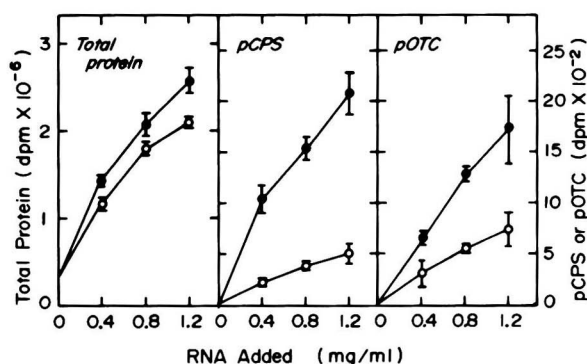


FIG. 5. Effect of dietary protein content on translatable mRNA levels for total protein, pCPS, and pOTC. Cell-free translation was performed with various concentrations of total hepatic RNA isolated from rats fed a 5% (○) or 60% (●) casein diet for 8 days. Synthesis of total protein, pCPS, and pOTC was measured as described under "Experimental Procedures." Each point represents the mean value of two animals, with the bars indicating the range.

TABLE I

Translatable mRNA levels for carbamyl phosphate synthetase I and ornithine transcarbamylase in rat liver

The data in Fig. 5 were used to calculate pCPS and pOTC synthesis, expressed as a percentage of total protein synthesis corrected for endogenous protein synthesis without exogenous RNA. Carbamyl phosphate synthetase I and ornithine transcarbamylase were assayed as described under "Experimental Procedures." Radial immunodiffusion was performed by placing 10 μ l of successively diluted liver

extracts on an agarose gel containing anti-rat carbamyl phosphate synthetase I (58 μ g/ml of immunoglobulin fraction) or anti-bovine ornithine transcarbamylase (25 μ g/ml of immunoglobulin fraction). The enzyme concentrations were calculated using the purified rat enzymes as standards. Values of enzyme activity and enzyme protein are means \pm S.D. (three animals).

Casein content	Enzyme activity		Enzyme protein		RNA added	pCPS/total protein		pOTC/total protein	
	Carbamyl-P synthetase I	Ornithine transcarbamylase	Carbamyl-P synthetase I	Ornithine transcarbamylase					
%	μ mol/h/g liver		mg/g liver		mg/ml	%		%	
5	535 \pm 117	19,700 \pm 1,700	4.0 \pm 0.4	0.50 \pm 0.03	0.4	0.028,	0.031	0.027,	0.051
					0.8	0.026,	0.026	0.037,	0.053
					1.2	0.024,	0.034	0.032,	0.045
						Mean = 0.028 \pm 0.004		0.041 \pm 0.011	
60	1,760 \pm 80	37,900 \pm 7,200	13.1 \pm 1.4	1.04 \pm 0.11	0.4	0.080,	0.116	0.054,	0.071
					0.8	0.090,	0.091	0.066,	0.083
					1.2	0.090,	0.096	0.066,	0.087
						Mean = 0.094 \pm 0.012		0.072 \pm 0.013	

TABLE II

Effects of mixing of RNA from rats kept on 5 and 60% casein diets on synthesis of total protein, pCPS, and pOTC

Cell-free translation was performed with total hepatic RNA from rats fed 5 or 60% casein diets for 8 days or with the mixture of equal amounts of RNA from both animals. The synthesis of total protein, pCPS, and pOTC was measured as described under "Experimental Procedures." Values of total protein synthesis are those corrected for endogenous protein synthesis without exogenous RNA, which was 0.31×10^6 dpm.

Casein content	RNA added	Incorporation of [35 S]methionine into:			pCPS/total protein	pOTC/total protein
		Total protein	pCPS	pOTC		
%	mg/ml	dpm $\times 10^{-6}$	dpm	dpm	%	%
5	0.4	0.90	156	242	0.017	0.027
	0.8	1.15	240	334	0.021	0.029
					Mean = 0.019 0.028	
60	0.4	1.13	690	568	0.061	0.050
	0.8	1.94	1550	1040	0.080	0.054
					Mean = 0.071 0.052	
5 + 60	0.2, each	1.17	527	488	0.045	0.042
	0.4, each	1.59	754	747	0.047	0.047
					Mean = 0.046 0.045	

was shorter (3.3 days) than in rats fed a protein-free diet (4.6 days). This observation, together with the findings in the present study, indicates that an increased protein intake results in an increased rate of both synthesis and degradation of carbamyl phosphate synthetase I, with the former predominating.

Similar findings have been observed in the tadpole. Thyroxine has been shown to increase the level of mRNA for carbamyl phosphate synthetase I and increased synthesis of the enzyme (30), and to decrease the half-life of the enzyme (31).

Effects of Fasting on Levels of Translatable mRNAs for the Enzymes—Schimke (12) reported that the levels of carbamyl phosphate synthetase I and ornithine transcarbamylase in liver of rats fasted for 7 days were of the order of 3- and 2.5-fold higher than in liver of rats fed a 15% casein diet. In the present study, using somewhat older rats of a different strain, fasting for 7 days resulted in increases (based on either enzyme activity or enzyme protein) of the order of 1.7- and 1.3-fold for

TABLE III

Effects of fasting on levels of carbamyl phosphate synthetase I and ornithine transcarbamylase in rat liver

Rats (200 to 210 g) were fed a commercial laboratory chow (protein content, 24%) *ad libitum* (control) or fasted for 7 days. Enzyme activities and enzyme concentrations of carbamyl phosphate synthetase I and ornithine transcarbamylase were measured as described under "Experimental Procedures." Values are means \pm S.D. (four animals). Changes in body weights of the control and fasted rats were $+30 \pm 7$ g and -63 ± 3 g, respectively. Liver weights of the control and fasted animals were 8.9 ± 0.7 g and 3.9 ± 0.3 g, respectively.

Rats	Enzyme activity		Enzyme protein	
	Carbamyl-P synthetase I	Ornithine transcarbamylase	Carbamyl-P synthetase I	Ornithine transcarbamylase
	μ mol/h/g liver		mg/g liver	
Control	923 \pm 58	28,300 \pm 800	7.45 \pm 1.06	0.82 \pm 0.11
Fasted	1,580 \pm 60	37,400 \pm 200	12.20 \pm 0.85	1.01 \pm 0.13

carbamyl phosphate synthetase I and ornithine transcarbamylase, respectively, compared to control animals fed a 24% casein diet (Table III).

In contrast to the findings in the present study on mRNA levels in rats fed high and low protein diets, the levels of translatable mRNA for carbamyl phosphate synthetase I and for ornithine transcarbamylase in fasted rats were 54 and 67%, respectively, of the values found in the control animals (Table IV). While the total translatable mRNA activity was also decreased in the fasted animals, the percentages of pCPS and pOTC synthesis of total protein synthesis remained essentially unchanged. Incubations with mixtures of equal amounts of hepatic RNA preparations from the control and fasted animals gave values for synthesis of total protein, pCPS, and pOTC which approximated the mean for the values obtained from separate incubations with each RNA preparation (data not shown). It thus appears that the increase in levels of carbamyl phosphate synthetase I and ornithine transcarbamylase in liver from fasted rats is not the result of an enhanced rate of synthesis determined by an increase in levels of mRNAs for these enzymes, but rather must be the result of a decreased rate of degradation of these enzymes.

Factors Determining the Increase in Urea Cycle Enzymes Following Increased Protein Intake, and Following Starvation—Schimke (11) observed that there was a coordinated response of the levels of all of the enzymes involved in urea biosynthesis to the level of dietary protein and to the level of

TABLE IV

Effects of fasting on translatable mRNA levels for carbamyl phosphate synthetase I and ornithine transcarbamylase in rat liver

Rats used were those described in the legend to Table III. Cell-free translation was performed with total hepatic RNA. Synthesis of total protein, pCPS, and pOTC was measured as described under "Experimental Procedures." Values are means \pm S.D. (four animals). Values of total protein synthesis are those corrected for endogenous protein synthesis without exogenous RNA which was 0.89×10^6 dpm.

Rats	RNA added mg/ml	Incorporation of [35 S]methionine into:			pCPS/total protein %	pOTC/total protein %
		Total protein dpm $\times 10^{-6}$	pCPS dpm	pOTC dpm		
Control	0.4	1.35 \pm 0.33	802 \pm 134	609 \pm 198	0.059	0.045
	0.8	2.24 \pm 0.18	1200 \pm 187	890 \pm 238	0.054	0.040
					Mean = 0.057	0.043
Fasted	0.4	0.63 \pm 0.14	371 \pm 191	353 \pm 79	0.059	0.056
	0.8	1.31 \pm 0.41	741 \pm 231	666 \pm 98	0.057	0.051
					Mean = 0.058	0.054

urea biosynthesis. In a study of the effects of fasting and protein-free diets, Schimke (12) found that the enzymes involved in urea biosynthesis were increased in the former and decreased in the latter.

The nature of the factor(s) operating to provide coordinated responses of the two mitochondrial enzymes (carbamyl phosphate synthetase I and ornithine transcarbamylase) and the three extramitochondrial enzymes (argininosuccinate synthetase (EC 6.3.4.5), argininosuccinase (EC 4.3.2.1), and arginase (EC 3.5.3.1)) involved in urea biosynthesis remains to be determined. While Schimke (32) provided some evidence for, and emphasized the importance of, differential rates of synthesis and degradation of the enzymes, the regulatory basis for this as yet incompletely understood.

Studies have been reported on the effect of dietary protein intake in the rat on the turnover of carbamyl phosphate synthetase I (29) and argininosuccinate synthetase (33), and on the effect of both starvation and different levels of protein intake on arginase turnover (32).

The findings from the present study showing an increase in mRNA for carbamyl phosphate synthetase I on a high protein diet and the findings by Nicoletti *et al.* (29) of a more rapid degradation rate under comparable dietary conditions indicate that increasing the protein content of the diet of rats results in an increase in both the rate of synthesis and of degradation, with the former predominating. While there are no data available on the effect of starvation on the turnover of carbamyl phosphate synthetase I in rats, the findings in the present study of a decreased level of mRNA for the enzyme indicate that the rate of degradation must be decreased as a result of starvation.

In a study of the effect of dietary protein intake on the turnover of rat liver argininosuccinate synthetase by Tsuda *et al.* (33), it was found that while both the rates of synthesis and degradation were involved in the regulation of the enzyme level during dietary transition, under steady state conditions, the rate of degradation was found to be unchanged while the rate of synthesis of the enzyme was determined to be the regulating factor.

In the case of arginase, Schimke (32) found that the observed increase in arginase in starved rats was the result of a decreased rate of degradation, while in the case of rats whose diet was changed from a high to a low protein intake, the increased levels of arginase was the result of an increased rate of degradation.

From the limited data available, it appears that as a result of starvation, the increase in levels of the enzymes involved in urea biosynthesis is the result of both a decrease in rate of synthesis (by decreasing mRNA levels) and of degradation,

with the latter predominating.

While the synthesis of both the mitochondrial and extra-mitochondrial enzymes involved in urea biosynthesis is under nucleo-cytoplasmic control, little is known about the proteolytic systems (and their regulation) affecting in particular the mitochondrial enzymes (34) and the basis for the selective degradation of liver proteins during starvation.

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