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Effect of branched-chain amino acids on albumin gene expression in the liver of galactosamine-treated rats

Masashi Kuwahata,* Tatsuzo Oka,* Kozo Asagi,† Hideaki Kohri,† Akinobu Kato,‡ and Yasuo Natori*

**Department of Nutrition, School of Medicine, The University of Tokushima, Tokushima 770, Japan; †Nutrition Research Laboratory, Otsuka Pharmaceutical Factory, Inc., Naruto 772, Japan; and ‡First Department of Internal Medicine, Iwate Medical University, Morioka 020, Japan*

Acute liver injury was induced in rats by the administration of galactosamine, which brought about inhibition of RNA synthesis and protein synthesis in the liver. The galactosamine-treated rats were infused with total parenteral nutrition solutions containing various proportions of branched-chain amino acids and the recovery from the galactosamine intoxication was followed by measuring albumin mRNA levels and polysome profiles. The levels of total cytoplasmic albumin mRNA were markedly decreased on day 2 after the galactosamine treatment, but the levels were restored to almost the control value by day 4 in all the groups irrespective of the content of branched-chain amino acids in the infusion solutions. On the contrary, the levels of β-actin mRNA changed in the opposite direction to those of albumin mRNA. It was also found that polysomes in the liver were extensively disaggregated on day 2 after the galactosamine treatment, but the polysome profiles returned to normal on day 4 in all the groups. However, when the levels of polysome-associated albumin mRNA were quantitated, we found that the extent of the recovery was dependent on the content of branched-chain amino acids in the infusion solutions; as the content of branched-chain amino acids increased, the level of polysome-associated albumin mRNA increased. These results suggest that the integration of albumin mRNA into functional polysomes in the liver is regulated by the supply of branched-chain amino acids. (J. Nutr. Biochem. 9:209–214, 1998) © *Elsevier Science Inc. 1998*

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Introduction

The expression of albumin gene is reduced in various liver diseases and the degree of reduction in the hepatic albumin mRNA level is generally correlated with the severity of the disease.[1] Acute liver cell injury can be induced by a number of agents with diverse physical, chemical, and biological characteristics. In 1968, Keppler et al.[2] reported that the administration of D-galactosamine to laboratory animals induced an injury of the liver that closely resembled human viral hepatitis in its morphological and functional features. There seem to be two specific biochemical lesions that are related to the hepatotoxic action of galactosamine; UTP deficiency and the accumulation of UDP-sugar nucleotides. The former may bring about inhibition of RNA synthesis and protein synthesis in the liver.[3]

Human and animal studies have indicated that the branched-chain amino acids (BCAAs), leucine, isoleucine and valine, play a unique role in total parenteral nutrition (TPN); BCAA-enriched hyperalimentation in stress has shown beneficial effects on nitrogen retention and protein metabolism.[4,5] The present study was designed to evaluate the effects of BCAA enrichment on the galactosamine-induced reduction of albumin gene expression in rat liver during parenteral nutrition.

Address correspondence and reprint requests to Dr. Yasuo Natori at Department of Nutrition, School of Medicine, The University of Tokushima, Kuramoto, Tokushima 770, Japan.
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Table 1 Composition of intravenous infusates

	Glucose	Standard amino acid formula	Hepatic amino acid formula	BCAA-enriched amino acid formula
Amino acids[1] (g/L)	0	31.3	32.8	36.6
Glucose (g/L)	250.0	219.0	217.5	213.5
Electrolytes[2]				
Vitamins[3]				

[1]Composition of amino acids is given in *Table 2*.
[2]Provided the following (mEq/L): Na^+, 75.0; K^+, 15.0; Cl^-, 75.0; Mg^{2+}, 10.0; Ca^{2+}, 7.5; PO_4^{2-}, 15.0; SO_4^{2-}, 10.0.
[3]Provided the following (mg/L): thiamin, 1.9; riboflavin, 2.3; pyridoxine, 2.5; cyanocobalamine, 0.003; nicotinamide, 25; folic acid, 0.25; panthenol, 8.8; biotin, 0.38; ascorbic acid, 62.5; retinol, 0.6; cholecalciferol, 0.003; tocopherol acetate, 6.3; and phytonadion, 1.3.

Methods and materials

Animals and infusion procedure

Male Sprague-Dawley rats, weighing about 200 g, were divided into four galactosamine-treated experimental groups of six animals each and prepared for continuous intravenous infusion as described previously.[6] In addition, galactosamine-untreated control groups (each consisted of three animals) were also infused with various TPN solutions.

Experimental design

During 4 days of postsurgical recovery period, a 0.9% saline was infused intravenously at the rate of 10 mL/day/rat. Animals were allowed free access to a commercial diet (CRF-1®, Oriental Co., Tokyo, Japan) and water during the recovery period. On the 5th day, designated as day 0 of the experiment, blood was withdrawn from the abdominal aorta of the animals of the control group under ether anesthesia, and then the liver was excised. D-galactosamine was injected intraperitoneally into rats of the experimental groups at a single dose of 800 mg/kg body weight, dissolved in 0.9% saline, and the animals were infused intravenously with a TPN solution at the rate of 200 mL/kg body weight/day. Food and drinking water were removed from the cages. The compositions of the infusion solutions are shown in *Tables 1* and *2*. Four different TPN solutions were employed, containing the same energy (1,000 kcal/L) and nitrogen (5 g/L) but different compositions of amino acids except the glucose group that contained no amino acids. The amino acid mixtures for the standard amino acid formula (Proteamin®) and hepatic amino acid formula (Aminoleban®) were obtained from Tanabe Pharmaceutical Co., Osaka, Japan and Otsuka Pharmaceutical Factory, Tokushima, Japan, respectively. BCAA-enriched amino acid formula was prepared in our laboratory by increasing the relative contents of BCAAs in the hepatic amino acid formula. The BCAA contents in the standard amino acid formula, the hepatic amino acid formula and BCAA-enriched amino acid formula were 21.3%, 35.5%, and 60.0%, respectively. On day 2 and day 4 of the experiment, the blood and liver samples were collected from three animals in each group for the subsequent analyses. Galactosamine-untreated control groups were killed on day 4, and the liver polysomal profiles were analyzed.

Analytical methods

Plasma samples were analyzed for the following parameters using an autoanalyzer (Hitachi 705, Hitachi, Tokyo, Japan): aspartate aminotransferase (AST) by the MDH-UV method, alanine aminotransferase (ALT) by the LDH-UV method, total protein by the biuret method, and albumin by the bromcresol green method.

Northern-blot hybridization

Total liver RNA and polysomal RNA were isolated and subjected to Northern-blot hybridization using ^{32}P-labeled rat albumin cDNA and β-actin cDNA as described previously.[7]

Analysis of polysomal profiles

Polysomes were prepared from the livers and analyzed by sucrose density gradient centrifugation as described previously.[8] Briefly, 2.0 A_{260} units of polysomal suspension was layered on the linear sucrose gradient (0.5 to 1.5 M) and then centrifuged 50,000 rpm for 1 hr in a Hitachi RPS-56T rotor. The ribosomal distribution was monitored by continuous flow through an ISCO absorbance monitor (Model UA-5) at 254 nm.

Preparation of polysomal RNA

Polysomes were prepared from the livers and polysomal RNA was isolated according to the method described by Palmiter et al.[9]

Table 2 Composition of amino acids in infusates (g/L)

	Standard amino acid formula	Hepatic amino acid formula	BCAA-enriched amino acid formula
L-Leu	3.1	4.5	8.5
L-Ile	1.6	3.7	6.9
L-Val	1.9	3.5	6.5
L-Met	1.2	0.4	0.3
L-Lys	2.2	2.4	1.7
L-Thr	1.4	1.8	1.3
L-Trp	0.5	0.3	0.2
L-Phe	2.7	0.4	0.3
L-Cys	0.07	0.13	0.09
L-Tyr	0.16	—	—
L-Arg	3.4	2.5	1.7
L-His	1.4	1.0	0.7
L-Ala	2.3	3.1	2.1
L-Asp	0.6	—	—
L-Glu	0.3	—	—
Gly	4.3	3.7	2.6
L-Pro	2.9	3.3	2.3
L-Ser	1.3	2.1	1.4
Total amino acids	31.33	32.83	36.59
Total nitrogen	5.0	5.0	5.0
BCAA (%)	21.3	35.5	60.0

Table 3 Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and albumin in the plasma of galactosamine-treated rats after 2 or 4 days of different parenteral nutrition

Group[1]	AST (units[2]/l)	ALT (units[2]/l)	Total protein (g/dl)	Albumin (g/dl)
I	98 ± 16	26 ± 1.5	4.7 ± 0.12	2.4 ± 0.17
II				
2 days	2660 ± 800	1110 ± 379	3.0 ± 0.33	1.8 ± 0.14
4 days	285 ± 30	110 ± 16	3.1 ± 0.88	1.7 ± 0.21
III				
2 days	3940 ± 930	1980 ± 615	2.7 ± 0.55	1.8 ± 0.36
4 days	151 ± 62*	36 ± 16**	3.7 ± 0.64	2.0 ± 0.31
IV				
2 days	3410 ± 619	1690 ± 471	2.6 ± 0.14	1.7 ± 0.02
4 days	119 ± 52**	29 ± 8.7**	3.5 ± 0.12	1.9 ± 0.06
V				
2 days	3160 ± 391	1440 ± 269	2.7 ± 0.35	1.6 ± 0.14
4 days	190 ± 65	46 ± 10**	3.0 ± 0.15	1.6 ± 0.06

[1]I. Control (no galactosamine treatment, day 0); II. Glucose (no amino acids); III. Standard amino acid formula; IV. Hepatic amino acid formula; V. BCAA-enriched amino acid formula.
[2]One unit is the enzyme activity to reduce the extinction at 340 nm at a rate of 0.001/min by the method of Karmen. Values are means ± SD.
*$P < 0.05$.
**$P < 0.01$ versus Group II −4 days.

Statistical analysis

Results were expressed as means ± SD. All data were analyzed by ANOVA plus Fisher's multiple comparison test. Results were considered significant when P was less than 0.05.

Results

Plasma proteins and enzymes

A single injection of D-galactosamine to rats, at a dosage of 800 mg/kg body weight, induced acute liver cell injury as evidenced by the rise of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the plasma of rats on day 2 after the injection (*Table 3*). After the injection of galactosamine, rats were nourished by intravenous infusion of TPN solutions containing various compositions of amino acids. The plasma aminotransferase activities returned to near normal levels by day 4. It can be seen in *Table 3* that the difference in the amino acid composition of TPN solutions did not appreciably affect the recoveries of the aminotransferase activities but the recovery was considerably poor in the animals infused with the amino acid-free TPN solution (Group II).

The concentrations of total proteins or albumin in the plasma were not significantly different among various experimental groups probably because the infusion period was short.

Expression of albumin and β-actin genes

After 2 or 4 days of parenteral nutrition in the galactosamine-treated rats, the livers were excised from the animals and the total cytoplasmic RNA isolated from the livers was subjected to Northern-blot analysis using albumin and β-actin cDNA probes (*Figure 1*). The levels of albumin mRNA were markedly decreased by the galactosamine treatment on day 2, but the levels were restored to almost the control value by day 4 in all the groups infused with the amino acid-containing TPN solutions. No difference was observed in the extent of the recovery in albumin mRNA among various groups infused with different compositions of amino acids. However, the animals, infused with the amino acid-free TPN solution, failed to recover the level of albumin mRNA.

On the contrary, the levels of β-actin mRNA changed in the opposite direction to those of albumin mRNA; the levels of β-actin mRNA were considerably increased by the galactosamine treatment on day 2, but the levels were restored to almost the control value by day 4 in all the groups except in the group infused with the amino acid-free TPN solution.

Polysome profiles

Protein synthesis in mammalian cells is carried out on polysomes where one strand of mRNA is associated with several ribosomes, each one carrying a peptide chain. It is generally accepted that the nutritional state dictates the number of ribosomes associated with the mRNA strand and, as a consequence, prescribes the rate of protein synthesis.[10,11]

On day 2 and day 4 after the galactosamine treatment, polysomes were prepared from the livers and analyzed by sucrose density gradient centrifugation. A representative profile in each experimental group is shown in *Figure 2*. Compared with the day-0 and galactosamine-untreated controls, extensive disaggregation of the liver polysomes was observed on day 2 after the galactosamine treatment. But, on day 4, the polysome profiles returned to normal in all the groups except Group II that was infused with the amino acid-free TPN solution. It seems that the supply of amino acids are necessary for the recovery of polysome profile after the galactosamine treatment. However, one should notice that the difference in amino acid composition in TPN solutions does not seem to influence the restoration of polysomes.

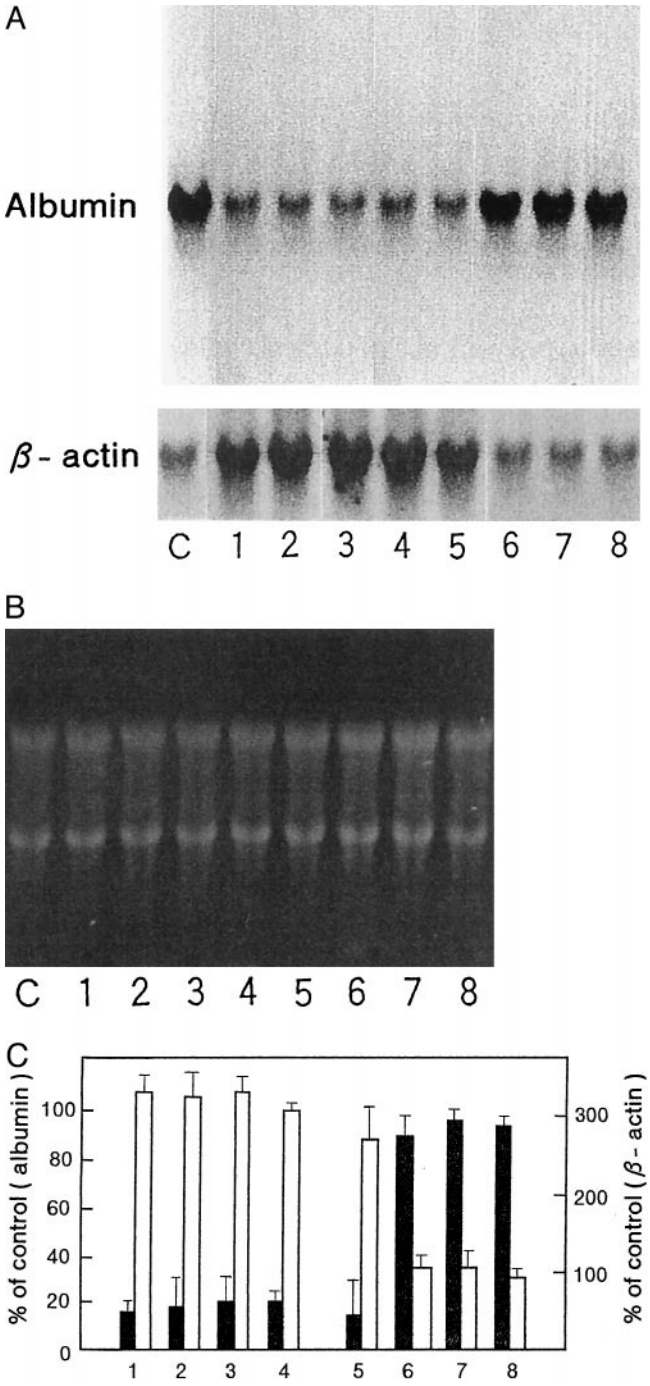

Figure 1 Effect of amino acid infusion on the levels of total albumin mRNA and β-actin mRNA in the liver of galactosamine-treated rats. The procedure for RNA isolation and Northern-blot hybridization are described in the section of Methods and materials. The gel shown is representative of three independent experiments. (A) Northern-blot analysis of total RNA from the livers of day-0 control (C) or galactosamine-treated rats (lanes 1–8). Lanes 1, 2, 3, and 4: after 2 days of parenteral nutrition with glucose (no amino acids), standard amino acid formula, hepatic amino acid formula, and BCAA-enriched amino acid formula, respectively. Lanes 5, 6, 7, and 8: after 4 days of parenteral nutrition with various formulae in the same order as above. (B) Ethidium bromide staining of ribosomal RNA as internal control of the amount of RNA loaded. (C) Densitometric estimation of albumin (closed bar) and β-actin (open bar) mRNA levels in Northern blots depicted in (A). The data are means ± SD.

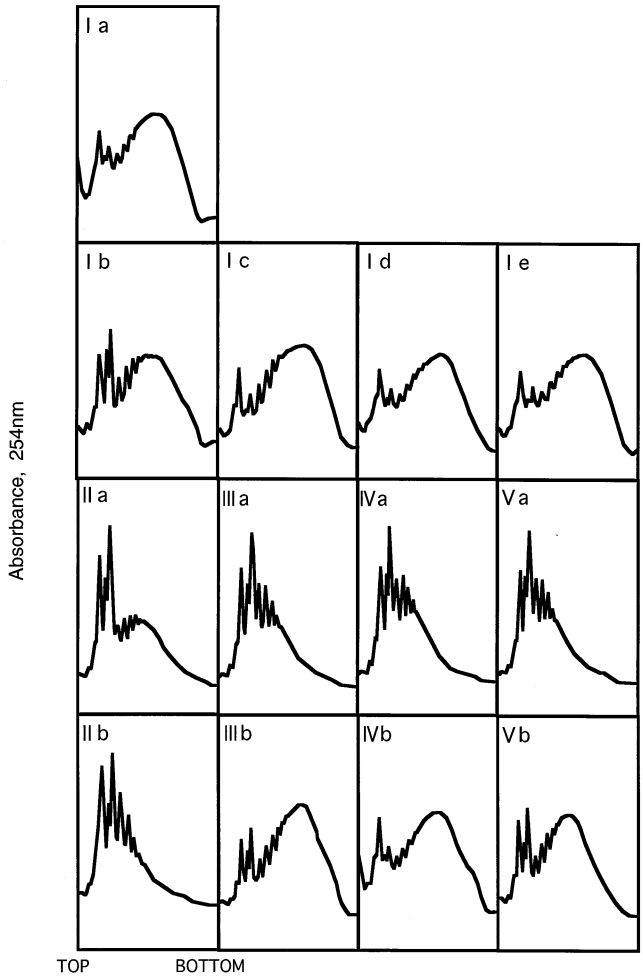

Figure 2 Sedimentation patterns of polysomes from the liver of galactosamine-treated rats after 2-day or 4-day infusion period of various test solutions. Sucrose density gradient centrifugation of the polysomes was performed as described in the section of Methods and materials. The pattern shown is representative of three independent experiments. I a, Control (day 0 of the experiment). I b, I c, I d, and I e; Controls (no galactosamine treatment) infused with glucose alone, standard amino acid formula, hepatic amino acid formula and BCAA-enriched amino acid formula, respectively, for 4 days. II a and II b, Glucose (no amino acids) for 2 days and 4 days, respectively; III a and III b, Standard amino acid formula for 2 days and 4 days, respectively; IV a and IV b, Hepatic amino acid formula for 2 days and 4 days, respectively; V a and V b, BCAA-enriched amino acid formula for 2 days and 4 days, respectively.

Polysome-associated albumin mRNA

We next prepared polysomes from the livers of the animals on day 2 and day 4 after the galactosamine treatment, isolated the polysomal RNA, and performed Northern-blot analysis for the estimation of albumin mRNA content. As shown in *Figure 3*, the levels of polysome-associated albumin mRNA were markedly reduced by the galactosamine treatment on day 2. On day 4, the level of albumin mRNA went down further in the amino acid-free group. Amino acid-supplemented groups showed recoveries in albumin mRNA, but the extent of the recovery was now different among groups infused with TPN solutions contain-

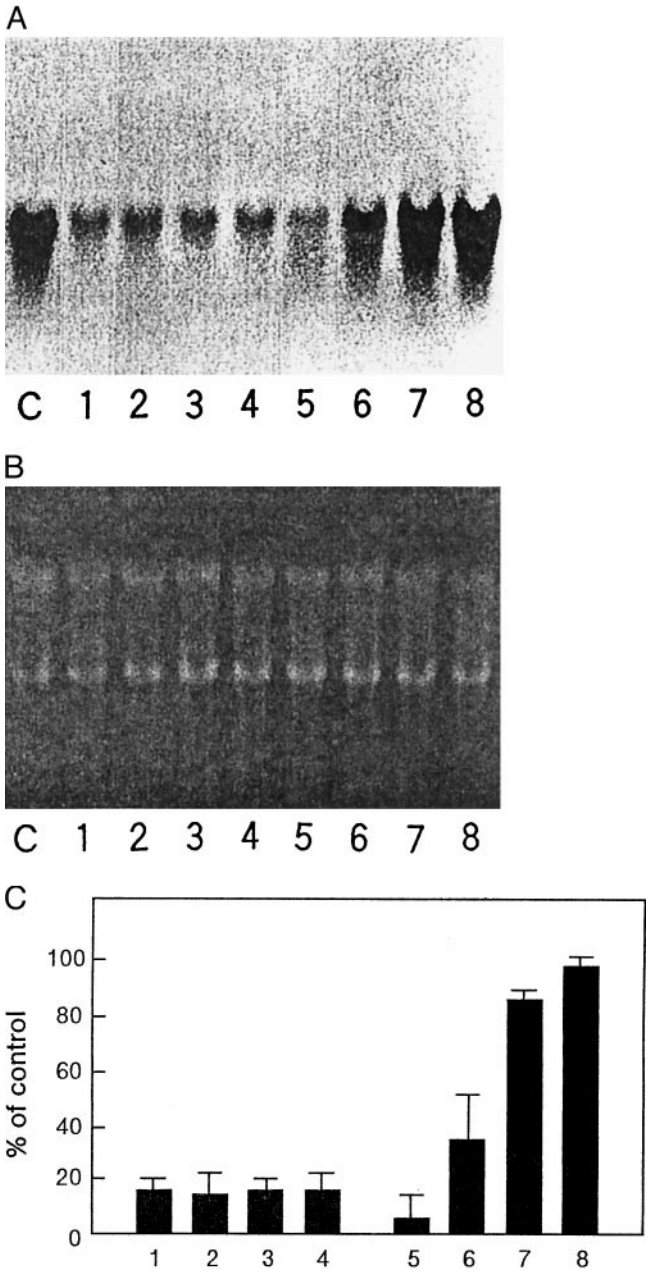

Figure 3 Effect of amino acid infusion on the level of polysome-associated albumin mRNA in the liver of galactosamine-treated rats. Polysomal RNA was isolated as described in the section of Methods and materials and Northern-blot analysis of albumin mRNA was performed as described in Figure 1. The gel shown is representative of three independent experiments. (A) Northern-blot analysis of polysomal RNA from the livers of day-0 control (C) or galactosamine-treated rats. The number of lanes corresponds to that in Figure 1. (B) Ethidium bromide staining of ribosomal RNA as internal control of the amount of ribosomal RNA loaded. (C) Densitometric estimation of albumin mRNA levels in Northern blots depicted in (A). The data are means ± SD.

ing different compositions of amino acids. It is evident that the recovery is dependent on the BCAA content in the amino acid formulae; as the BCAA content in the TPN solutions increases, the content of polysome-associated albumin mRNA increases.

Discussion

It is well known that reduction of the plasma BCAA/aromatic amino acid ratio (Fischer's ratio) is related to the pathogenesis of hepatic encephalopathy in patients with cirrhosis.[12] Infusion of a BCAA-enriched solution has been shown to improve hepatic encephalopathy in such patients.[13] BCAA also appears to act favorably in albumin metabolism. Oral supplementation of BCAA has been reported to improve the production rate of albumin in patients with decompensated cirrhosis.[14] A similar phenomenon has been reported with post-traumatic sepsis. Chiarla et al.[15] have reported that plasma BCAA concentrations are decreased in patients with sepsis, and the administration of BCAAs induces a rapid rise in plasma albumin levels. However, the mechanism whereby BCAAs improves albumin synthesis in the liver has remained unknown.

In the present study, acute liver cell injury was induced by the administration of galactosamine. Galactosamine causes inhibition of RNA synthesis and protein synthesis in the liver as a result of a reduction in the intracellar UTP concentration.[3] Presently observed decrease in total cytoplasmic albumin mRNA level and disaggregation of polysomes in the liver on day 2 after galactosamine administration (*Figures 1 and 2*) are in line with the previously described mode of action of galactosamine.[3] On day 4 after the galactosamine treatment, both albumin mRNA level and polysome disaggregation were recovered to the control levels in all the groups except in the animals that were infused with amino acid-free TPN solution (*Figures 1 and 2*).

After galactosamine treatment, the levels of β-actin mRNA changed in the opposite direction to those of albumin mRNA. The levels of β-actin mRNA were considerably increased by galactosamine treatment on day 2, but the levels were restored to almost the control value by day 4 in all the groups except in the group infused with amino acid-free TPN solution (*Figure 1*). It has been reported that β-actin mRNA level increases during regeneration and repair of rat liver after carbon tetrachloride-induced liver damage.[16] The presently observed changes of β-actin and albumin mRNA levels in an anti-parallel manner seem to reflect the states of injury and recovery processes in the liver after the galactosamine treatment.

Regarding the three different iso-nitrogenous amino acid formulae employed in the present study, the standard amino acid formula (Group III) is the conventional amino acid mixture, whereas the hepatic amino acid formula (Group IV) has been developed specifically for the treatment of hepatic encephalopathy. The BCAA-enriched amino acid formula (Group V) was prepared in our laboratory by almost doubling the relative BCAA contents in the hepatic amino acid formula at the cost of other amino acids.

The supply of amino acids was essential in the recovery process of albumin mRNA and ribosome aggregation from galactosamine intoxication. The recovery of plasma aminotransferase activities after galactosamine treatment was also facilitated by the inclusion of amino acids in TPN solutions (*Table 3*).

The recoveries of total cytoplasmic albumin mRNA level and ribosome aggregation from galactosamine intoxication were not influenced by the different BCAA contents in the

TPN solutions presently used. However, the recovery of polysome-associated albumin mRNA, on day 4 after the galactosamine treatment, was remarkably dependent on the BCAA content in the amino acid formulae; as the BCAA content in the TPN solutions increased, the content of polysome-associated albumin mRNA increased (*Figure 3*). Inasmuch as polysome profiles and total cytoplasmic albumin mRNA were almost fully recovered to the control levels by day 4 (*Figures 1 and 2*), the integration of albumin mRNA into polysome structure seems to be dependent on BCAA contents in TPN solutions.

An earlier report of Montoya et al.[17] has shown that albumin synthesis and secretion in cultured rat hepatocytes are specifically stimulated by supplementation of culture medium with BCAAs. A recent work of Okuno et al.[18] has extended this observation to show that the synthesis and secretion of albumin in the primary culture of rat hepatocytes are stimulated by increasing the BCAA/aromatic amino acid ratio in the culture medium. Because the stimulation occurred without the change of albumin mRNA level, BCAAs were presumed to act at the translational level. Our present results are consistent with these previous reports and emphasize the importance of BCAAs in the process of integration of albumin mRNA into functional polysome.

It has been reported that 97 to 98% of albumin mRNA sequences in normal rat liver are present in membrane-bound polysomes.[19] When rats are fasted for 24 to 30 hr, albumin mRNA sequences are released from membrane-bound polysomes to enter the free cytosol fraction and refeeding a mixture of amino acids restores albumin mRNA to membrane-bound polysomes.[20] Whether or not BCAAs play a unique role in the restoration process is not known.

Transcription of albumin gene is known to be influenced by protein nutrition status and we have recently reported that modulation of albumin gene expression by amino acid supply in rat liver is mediated through intracellular concentration of pyridoxal 5′-phosphate.[21] The molecular mechanism of the translational control of albumin synthesis by amino acids, particularly by BCAAs, is yet to be elucidated. It also remains to be established whether other acute liver proteins are affected similarly to albumin by BCAAs. The galactosamine-induced liver cell injury, employed here, may offer an excellent system to study these problems.

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