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Modulation of albumin gene expression by amino acid supply in rat liver is mediated through intracellular concentration of pyridoxal 5′-phosphate

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Rats were nourished by infusion of total parenteral nutrition solutions containing 0% or 3.3% amino acids for 7 days. The level of albumin mRNA in the liver of amino acid-infused rats was found to be several fold higher than that in the liver of amino acid-depleted rats. Expression of albumin gene is known to be regulated by tissue-specific transcription factors such as HNF-1 and C/EBP. We determined the binding activities of liver nuclear extracts to the HNF-1- and C/EBP-binding sites by gel mobility-shift assay and found that the activities of the extract prepared from liver of amino acid-infused rats were greater than those of amino acid-depleted rats. In view of our recent finding that vitamin B_6 modulates albumin gene expression through inactivation of tissue-specific transcription factors by direct interaction with pyridoxal phosphate, we determined the intracellular concentrations of vitamin B_6 derivatives. We found that the concentration of pyridoxal phosphate in the liver of amino acid-infused rats was decreased to almost half of that of amino acid-depleted rats, whereas the concentration of pyridoxamine phosphate was increased in the opposite direction. These observations suggest that an increase in albumin mRNA level in the liver of amino acid-infused rats may be caused by a decrease in intracellular concentration of pyridoxal phosphate, which in turn relieves inactivation of tissue-specific transcription factors. (J. Nutr. Biochem. 8:211–216, 1997) © *Elsevier Science Inc. 1997*

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Introduction

The signals regulating gene expression in prokaryotes and lower eukaryotes in response to nutrient variation are well known and characterized, but much less is known about the response of higher eukaryotes to nutrient deprivation.[1,2] Because protein synthesis plays the central role in cellular function, some intricate mechanisms must exist to detect and respond to amino acid deprivation.

A recent study in our laboratory has shown that depletion of amino acid supply to rats during total parenteral nutrition (TPN) does not alter the fractional synthesis rate of liver domestic proteins, but decreases the synthesis rate of plasma proteins, particularly albumin.[3] However, little is known about the molecular mechanism whereby the level of amino acid supply to animals differentially influences the synthesis rates of domestic proteins and plasma proteins in the liver.

We have recently shown that vitamin B_6 modulates albumin gene expression in rat liver through a novel mechanism that involves inactivation of tissue-specific

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transcription factors by direct interaction with pyridoxal 5′-phosphate (PLP).[4] We report here that amino acid deprivation in rats brings about an increase in the PLP concentration in the liver with concomitant inactivation of tissue-specific transcription factors such as HNF-1 and C/EBP, indicating that amino acid-dependent modulation of albumin gene expression is mediated, at least in part, through the changes in the intracellular PLP concentration.

Methods and materials

Animals and infusion procedure

Male Wistar rats, weighing about 200 g, were divided into two groups of 5 animals each and prepared for continuous intravenous infusion as described previously.[3] The compositions of the infusion solutions, containing 0% or 3.3% amino acids were also described.[3] The infusion solutions also contained a vitamin mixture including pyridoxine (1.5 mg/L). After 7 days of infusion, animals were killed by a blow to the head and decapitated before isolation of the liver.

For the experiments involving the administration of a large dose of vitamin B_6, another set of rats were infused with 0% amino acid (5 animals) or 3.3% amino acids (15 animals) for 7 days. The rats infused with 3.3% amino acids were divided into three groups of 5 animals each; the first group received an intraperitoneal injection of pyridoxine (10 mg/100 g body weight) in an isotonic 0.9% NaCl solution (1 mL), the second group received an isotonic NaCl solution alone, and the third group received no injection. All the animals were killed 5 hr later and the livers were excised for the determination of albumin mRNA levels.

Northern-blot hybridization

Total RNA was isolated from the livers and subjected to Northern-blot hybridization using ^{32}P-labeled rat albumin cDNA as described previously.[4] The autoradiographs were scanned densitometrically to determine mRNA levels quantitatively.

Nuclear-transcription assay

Nuclei were isolated from the livers and subjected to run-on transcription assay as described previously.[4]

Gel mobility-shift assay

Two oligonucleotides (CTGTAGATCATTAACCA and GTATG TTTGCCATCTGG), representing binding sites of HNF-1 and C/EBP respectively,[5] were synthesized on an Applied Biosystems synthesizer, and double-stranded oligonucleotides were labeled with ^{32}P at the 5′ end by T_4 polynucleotide kinase. Mobility-shift assay, using nuclear-extract protein, was performed as described previously.[4]

Western-blot analysis

Western-blot analysis of C/EBP content in nuclear extracts was performed as described previously.[4]

Determination of vitamin B_6 derivatives

The concentrations of various vitamin B_6 derivatives in the liver were determined by a reversed-phase HPLC method, developed in the laboratory of one of the present authors (H.T.).[6]

Determination of proteins

Total protein in the plasma was determined by the biuret method.[7] Albumin was measured with bromocresol green.[8]

Results

Increase in albumin mRNA in the liver of amino acid-infused rats

Rats were nourished for 7 days by intravenous infusion of isocaloric TPN solutions containing 0% or 3.3% amino acids. During 7 days of parenteral nutrition, the animals that received the solution containing 3.3% amino acids gained 15 g in body weight, whereas the 0% amino acid group showed a 24-g weight loss. The concentration of total plasma protein and albumin in the 3.3% amino acid group was 45 ± 2 g/L and 37 ± 2 g/L, respectively. In the 0% amino acid group, the concentration of total plasma protein and albumin was decreased to 36 ± 1 g/L and 27 ± 2 g/L, respectively.

After 7 days of parenteral nutrition, the livers were excised from the animals and total RNA isolated from the livers was subjected to Northern-blot analysis using albumin cDNA probe (*Figure 1*). The concentration of albumin mRNA (2.2 kb) in the liver of 3.3% amino acid group, determined by densitometric tracing, was about 5 fold higher than that in 0% amino acid group. The higher-molecular-weight band, seen in 3.3% amino acid group, seemed to be a nonspecific contamination; the same band could be recognized in the amino acid-depleted group after longer exposure.

Elevation of albumin mRNA is because of increased transcriptional rate

To investigate whether an increase in transcriptional rate might account for the elevated level of albumin mRNA, we performed a nuclear run-on assay.

As shown in *Figure 2,* the rate of transcription of the albumin gene was enhanced 5 fold in 3.3% amino acid group, indicating that the higher level of albumin mRNA in the liver of amino acid-infused rats could be because of the enhanced rate of transcription. Transcription of β-actin gene, an internal control, was found to be unchanged by the amino-acid infusion. When α-amanitin, an inhibitor of RNA polymerase II, was added to the reaction mixture (2 μg/mL), transcription of the albumin gene was almost completely inhibited, suggesting that the transcripts result from RNA polymerase II activity.

Increase in binding activity of nuclear extract to HNF-1- and C/EBP-binding sites in albumin gene by amino-acid infusion

There is a hierarchy of importance of the various transcription factors regulating albumin gene expression in the liver; the HNF-1- and C/EBP-binding sites activate transcription more strongly than the other sites.[9] We synthesized two oligonucleotides, CTGTAGATCATTAACCA (correspond-

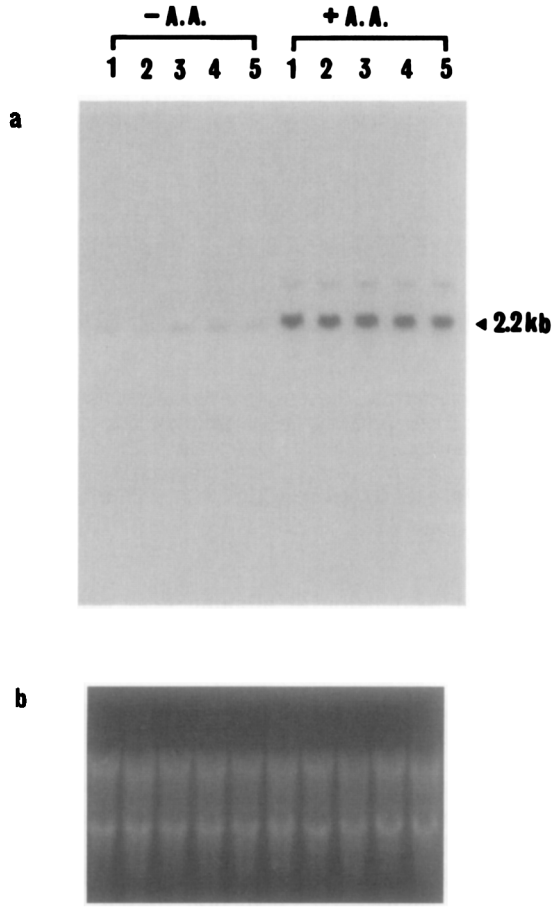

Figure 1 Effect of amino acid infusion on the level of albumin mRNA in rat liver. The procedure for RNA isolation and Northern-blot hybridization are described in the section of Methods and materials. *a*, Northern-blot analysis of total RNA from the livers of amino acid-depleted (−A.A.) and amino acid-infused (+A.A.) rats with albumin cDNA probe. Each lane represents RNA sample from the liver of an individual rat. *b*, Ethidium bromide staining of ribosomal RNA as internal control of the amount of RNA loaded.

ing to nucleotides −44 to −60 of rat albumin gene) and GTATGTTTGCCATCTGG (nucleotides −151 to −167 of rat albumin gene), which interact with HNF-1 and C/EBP respectively.[5] We then assayed the binding activities of nuclear extracts to each of these oligonucleotides by mobility-shift analysis. *Figure 3a* shows that the binding activity of nuclear extract prepared from the liver of amino acid-infused rats (lanes 6–10 and lanes 16–20) to both nucleotides was greater than that of amino acid-depleted rats (lanes 1–5 and lanes 11–15). The sequence specificity of the binding was verified by the addition of 50 to 100 fold molar excess of unlabeled competitor oligonucleotides. Both unlabelled competitors blocked the formation of the complex between nuclear extract and test DNA probes (*Figure 3b*, lanes 1–3 and lanes 7–9). The mutant oligonucleotide competitors, on the contrary, did not affect the binding activity (*Figure 3b*, lanes 4–6 and lanes 10–12). This result indicates that the concentration and/or DNA-binding activity of HNF-1 and C/EBP were elevated in the liver of amino acid-infused rats.

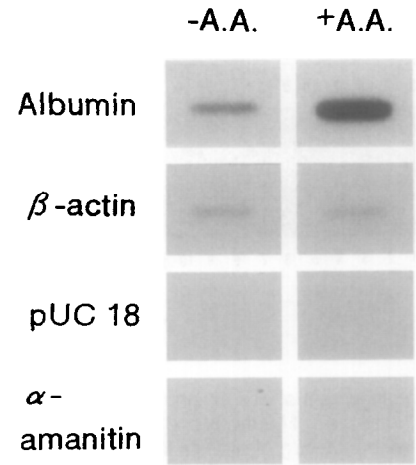

Figure 2 Effect of amino acid infusion on transcription of albumin gene in rat liver. The procedure for nuclear run-on assay is described in the section of Methods and materials. β-Actin was used as an internal control. A pUC18 cDNA is shown as a negative control. α-Amanitin (2 μg/mL) was added as an inhibitor of RNA polymerase II. The gel shown is representative of five independent experiments.

Content of C/EBP is unchanged in the liver of amino acid-infused rats

To estimate the relative abundance of C/EBP in nuclear extracts, Western-blot analysis was performed using C/EBP-specific antibody. As illustrated in *Figure 4*, a major band of approx. 42 kDa and a minor band of 32 kDa were observed. The latter probably represents a truncated form of C/EBP, a result of proteolytic degradation during extraction. The fact that the intensities of the bands with nuclear extracts from amino acid-infused group and amino acid-depleted group were essentially the same suggests that the liver in amino acid-depleted group contains almost the same concentration of C/EBP as the liver in amino acid-infused group. We therefore conclude that the high DNA-binding activity of the nuclear extract from the amino acid-infused group, shown in *Figure 3*, is probably because of activation of the transcription factor.

Decrease in PLP concentration and increase in PMP concentration in the liver by amino-acid infusion

We have recently proposed that the intracellular level of PLP modulates expression of albumin gene by modulating the activities of tissue-specific DNA-binding proteins such as HNF-1 and C/EBP.[4] We therefore determined the intracellular concentrations of PLP and its analogues in the livers of amino acid-infused and amino acid-depleted rats.

As shown in *Table 1*, the PLP concentration in the liver of the amino acid-infused rats was decreased to almost a half that of the amino acid-depleted rats while pyridoxamine 5′-phosphate (PMP) concentration was significantly increased. The PLP/PMP ratio in the amino acid-infused rat-liver was therefore less than a half that of the amino acid-depleted liver. The concentration of minor vitamin B_6 derivatives such as 4-pyridoxic acid (PIC) and pyridoxal (PL) were essentially unchanged by amino-acid infusion.

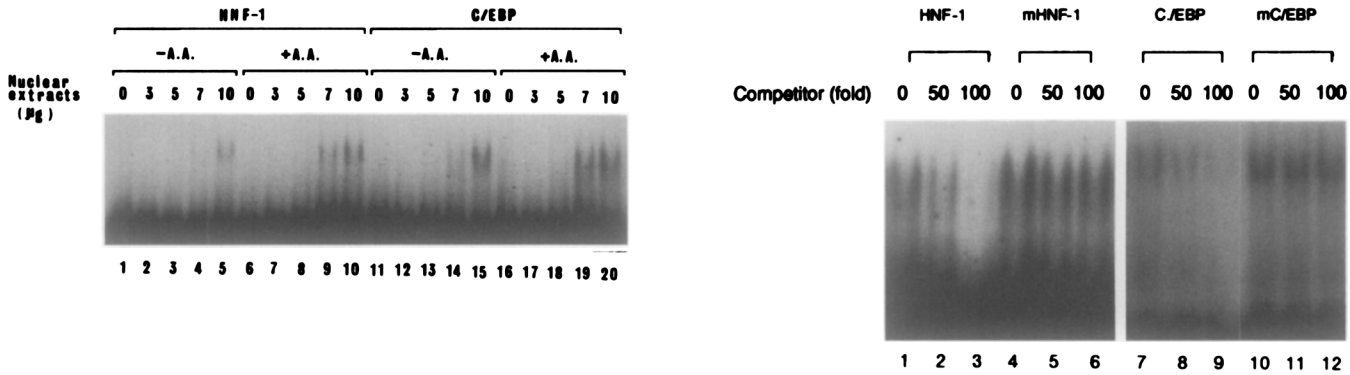

Figure 3 Enhancement of HNF-1- and C/EBP-binding activities in the liver of amino acid-infused rats. The procedure for gel mobility-shift assay is described in the section of Methods and materials. *a*, Mobility-shift assays of HNF-1-binding (lanes 1–10) and C/EBP-binding (lanes 11–20) proteins in nuclear extract (0–10 μg of protein) from the livers of amino acid-depleted (−A.A.) and amino acid-infused (+A.A.) rats. *b*, Effect of addition of competitor of oligonucleotides. Nuclear extract (10 μg of protein) from amino acid-infused rat liver was assayed in the presence of 50 and 100 fold molar excess of unlabeled competitor oligonucleotides and the mutant oligonucleotides (mHNF-1; CTGTAGATCACCGGCCA, mC/EBP; GTATGCCC GCCATCTGGG) as a control.

Administration of vitamin B_6 suppresses elevation of albumin mRNA by amino-acid infusion

To prove that elevation of albumin mRNA in the amino acid-infused rat-liver is because of the decrease in PLP concentration, the effect of vitamin B_6 administration on albumin mRNA level was investigated. We injected pyridoxine (10 mg/100 g body weight in 1 mL saline) to the amino acid-infused rats and found that the increased albumin mRNA level was reduced to less than half within 5 hr of the vitamin administration (*Figure 5*). This observation suggests that modulation of albumin gene expression by amino acid supply is indeed mediated through changes in the intracellular concentration of PLP.

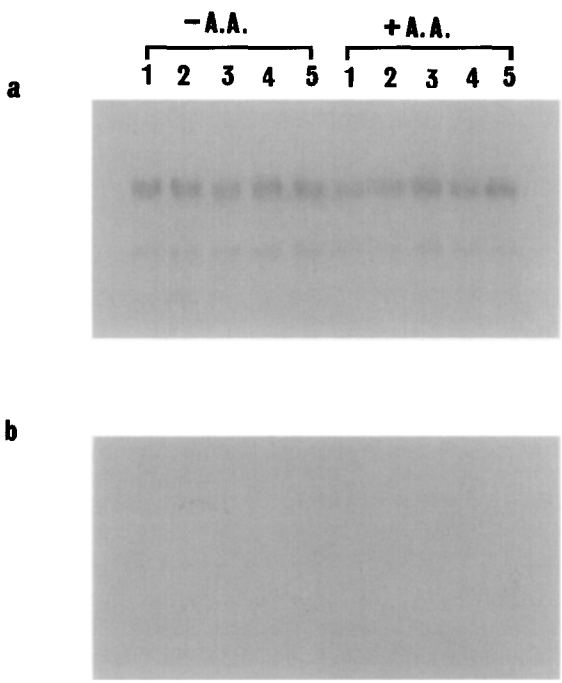

Figure 4 Western-blot analysis of C/EBP in nuclear extracts prepared from the livers of amino acid-depleted and amino acid-infused rats. *a*, The procedure for Western-blot analysis is described in the section of Methods and materials. Each lane represents nuclear extract from the liver of an individual rat in amino acid-depleted (−A.A.) or amino acid-infused (+A.A.) group. *b*, Pre-immune serum was used as control.

Discussion

Nutritional studies involving protein deficiency have often been hampered by a difficulty in controlling the amount of experimental diet taken by the animals. If removal of protein from the diet is associated with loss of appetite and reduced food intake, the distinction of the sequelae of protein deprivation from those of starvation poses a difficult problem. An earlier study by Sakuma et al.[10] has indicated that transcription of albumin mRNA in rat liver is influenced not only by the level of dietary protein, but also by the level of energy intake.

The technique of TPN offers a unique opportunity to rigorously modulate the quality and quantity of nutrient influx into animals, abolishing individual and diurnal variations in protein metabolism because of variations in quantity and timing of food intake. In the present study, animals were infused with solutions containing the same energy and 0% or 3.3% amino acids. These amino acid levels may correspond to protein-free and normal protein diets, respectively, in the feeding experiments.

In the present study, we observed that albumin mRNA concentration in the liver of 3.3% amino acid group was several fold higher than that in the 0% amino acid group. The nuclear run-on assay indicated that the higher level of albumin mRNA in the amino acid-infused group was caused by the enhanced rate of transcription.

Expression of albumin gene is known to be regulated by tissue-specific transcription factors such as HNF-1 and C/EBP.[5] We have now found that the binding activity of liver nuclear extract to HNF-1- and C/EBP-binding sites in the albumin gene is higher in the amino acid-infused rats than in the amino acid-depleted rats. As the concentrations of C/EBP in the nuclear extracts, determined by Western blotting, were essentially the same for both amino acid-infused and amino acid-depleted animals, we concluded that the lower DNA-binding activity of the nuclear extract from

Table 1 Effect of amino acid infusion on the concentrations of vitamin B_6 derivatives in rat liver

Amino acid	(nmol/g of liver)				
	PIC	PL	PLP	PMP	PLP/PMP
0%	0.690 ± 0.129	3.34 ± 0.65	20.3 ± 1.9	9.9 ± 1.0	2.05 ± 0.12
3.3%	0.585 ± 0.192	2.76 ± 0.38	11.5 ± 3.4*	13.0 ± 1.5*	0.89 ± 0.21**

Experimental details are described in the section of Methods and Materials. Rats were nourished for 7 days by intravenous infusion of isocaloric TPN solutions containing 0% or 3.3% amino acids. Concentrations of vitamin B_6 derivatives in the liver were determined by an HPLC method. Values are means ± SD for 5 rats.
*$P < 0.01$, **$P < 0.001$ vs. 0% amino acid group.

the liver of amino acid-depleted animals was caused by inactivation of C/EBP rather than a decrease in its concentration. Although we have not performed a similar experiment with HNF-1 because of unavailability of HNF-1-specific antibody, we expect that HNF-1 will behave similarly to C/EBP.

We have recently reported that PLP modulates expression of albumin gene in rat liver through a novel mechanism that involves inactivation of tissue-specific transcription factors.[4] The determination of the intracellular concentrations of vitamin B_6 derivatives showed that the PLP concentration in the liver of amino acid-infused rats was decreased to almost a half that of amino acid-depleted rats, whereas the PMP concentration was increased in the opposite direction. That the change in PLP concentration is causatively related to the change in albumin gene expression is indicated by attenuation of albumin mRNA level by the administration of pyridoxine to the amino acid-infused rats. We observed that the albumin mRNA level was reduced to less than half within 5 hr of the pyridoxine administration. A similar kinetic of the pyridoxine-induced albumin mRNA decay was previously observed in the vitamin B_6-deficient rats.[4] These results suggest that an increase in albumin mRNA level in the liver of amino acid-infused rats may be caused by a decrease in intracellular concentration of PLP. We have proposed that PLP interacts with tissue-specific transcription factors by forming a Schiff base between its aldehyde group and primary amino groups, most commonly the ϵ-amino group of lysine residues, resulting in a decreased DNA-binding activity of the transcription factors.[4]

The question as to how amino acid infusion decreases hepatic PLP concentration may be raised. Amino acids, transported into hepatic cells, will undergo transamination reactions catalyzed by aminotransferases. Since PLP is the coenzyme of all the aminotransferases and enzyme-bound PLP is converted to PMP during transamination, the continuous influx of amino acids will decrease PLP concentration and elevates PMP concentration in the steady state.

As the coenzyme of many amino acid-metabolizing enzymes, PLP has been known to be intimately involved in amino acid metabolism. Apart from its role as coenzyme, our recent studies have shown that PLP serves as a modulator of gene expression in the liver by inactivating RNA polymerase[11] and tissue-specific transcription factors such as HNF-1, C/EBP,[4] and glucocorticoid receptor.[12] The present study adds another possibility to the physiological

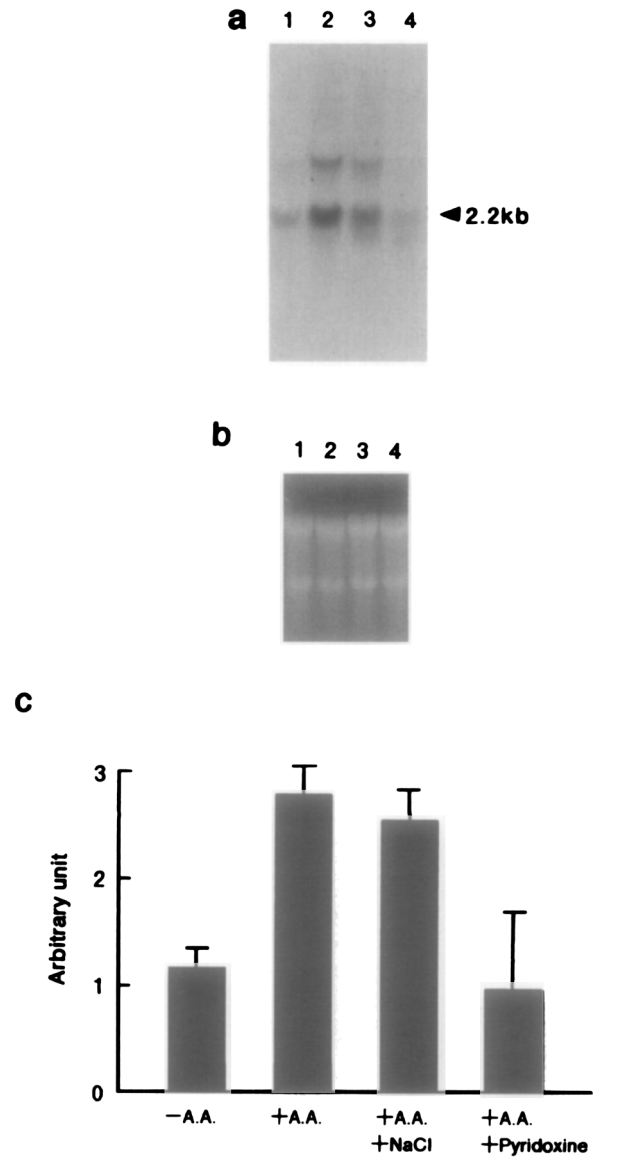

Figure 5 Effect of the administration of pyridoxine on albumin mRNA expression in rat liver. Treatment of rats and Northern-blot analysis for albumin mRNA are described in the section of Methods and materials. *a*, Northern-blot analysis of total RNA from the livers of amino acid-depleted rats (lane 1), amino acid-infused rats (lane 2), amino acid-infused rats 5 hr after the administration of isotonic NaCl (lane 3), and amino acid-infused rats 5 hr after the administration of pyridoxine (10 mg/100 g body weight) in isotonic NaCl (lane 4) with albumin cDNA probe. *b*, Ethidium bromide staining of ribosomal RNA as internal control of the amount of RNA loaded. *c*, Densitometric estimation of albumin mRNA levels in Northern blots depicted in (*a*). The data are means ± SD for 5 rats.

role of PLP as a mediator of gene regulation in higher animals by amino acid supply.

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