Concentrations of Asparagine in Tissues of Prepubertal Rats after Enzymic or Dietary Depletion of Asparagine

By DAVID L. FRANKEL, HERBERT WELLS and LOUIS CHARLES FILLIOS

Department of Biochemistry, Division of Basic Sciences, Boston University Medical Center,
100 East Newton Street, Boston, Mass. 02118, U.S.A.

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Growth of weanling rats was significantly depressed after 8 days of asparagine depletion produced by dietary means or by asparaginase treatment. Moreover, the concentration of free asparagine was significantly lowered in forebrain, skeletal muscle, liver, kidney, spleen and small intestines 3 h after an asparaginase injection, but remained lowered only in forebrain and skeletal muscle after 8 days of enzymic or dietary depletion of asparagine.

Growth of neonatal and weanling rats is affected by specific amino acid deficiencies. In addition to the established evidence with regard to recognized essential amino acids, Breuer *et al.* (1964) indicated that asparagine also may be essential during certain phases of growth in weanling rats. The high content of asparagine (4%) in milk protein suggests also a special requirement in suckling rats. Other studies have emphasized the importance of asparagine for DNA replication, cell division and RNA and protein synthesis in the regenerating liver, lymphocytes and various strains of asparaginase-sensitive acute lymphatic leukaemias and lymphomas in mice, rats and man (Becker & Broome, 1967; Becker *et al.*, 1970; Miura *et al.*, 1970; Mukherjee, 1970; Ellem *et al.*, 1970). In some instances intracellular depletion of asparagine or deficits of asparagine synthetase activity have been reported for such systems. Ottaway (1972) has demonstrated the selective lowering of tissue asparagine concentrations in adult female rats treated with growth hormone, a finding suggesting that decreased asparagine concentrations may be limiting in such hormonally stimulated growth.

Materials and methods

Prepubertal male Sprague-Dawley rats (Charles River Breeding Laboratories, North Wilmington, Mass., U.S.A.) were housed individually and fed on specific diets *ad libitum*; daily feed intakes and body weights were recorded. Chronically (8-day) L-asparaginase-treated (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.) rats were pair-fed with controls receiving saline (0.9% NaCl) injections. Amino acids in the semi-synthetic diets used were derived either from casein or from an asparagine-, glutamine- and aspartate-free amino acid mixture making up 20% (w/w) of the diet; the other ingredients of this diet were as previously reported (Fillios & Yokono, 1968). [The composition of the

amino acid mixture is as follows (in %, w/w): L-arginine (free base), 4.5; L-histidine hydrochloride monohydrate, 4.0; L-leucine, 8.5; L-isoleucine, 6.5; L-lysine hydrochloride, 9.5; DL-methionine, 4.0; L-phenylalanine, 6.0; DL-threonine (allothreonine-free), 6.5; DL-tryptophan, 2.0; DL-valine, 11.0; DL-alanine, 2.5; L-glutamic acid (free base), 14.0; glycine, 3.0; L-proline, 2.5; DL-serine, 2.5; L-tyrosine, 5.0; NaHCO₃, 8.0. This mixture made up 19.2% (w/w) of the total diet; either asparagine or diammonium citrate made the additional 0.8% of the diet.] For the present study we found that the above amino acid mixture was suitable since it compared very favourably in our laboratory (in terms of growth responses in 21-day-old rats) with those reported by others (Breuer *et al.*, 1964; Rogers *et al.*, 1970).

All the rats were 32 days of age at the time they were killed. The four control animals in the first study were fed on a purified diet containing casein as the source of protein and received intraperitoneal injections of saline daily during an 8-day period before they were killed; another group of four received exactly the same treatment but 3 h before being killed received a single intraperitoneal injection of 60 units of asparaginase. Another group of rats received daily intraperitoneal injections of asparaginase during this 8-day period and then were killed. In the second experiment the controls received the semi-synthetic diet with the described asparagine-free amino acid mixture as the only source of amino acids during the 8 days before they were killed; another group received the same diet but supplemented with 0.8% of asparagine during the same 8-day period.

After total body weight had been measured, the small intestine, lower-hind-limb skeletal muscle, kidney, testes, spleen, forebrain and liver were dissected and placed in culture tubes containing 3 ml of boiling deionized water (Shurr *et al.*, 1950). Approx. 0.5 g segments of small intestine were quickly dissected and any residual food was removed before the

tissues were placed in boiling water; 0.5 g segments of other tissues were also cut; for skeletal muscle the tissue was dissected from the lower hind-limb after this had been cut transversely at the knee. All tissue samples were quick-frozen and stored before subsequent procedures.

Tissues were analysed for asparagine and aspartate by using the enzymic method described by Kojima & Wacker (1969), as adapted for tissue analyses by Cooney *et al.* (1970).

Results and discussion

At the end of 8 days the percentage gain in body weight was less both for rats fed on an asparagine (and glutamine and aspartate)-free diet and for those treated daily with asparaginase when compared with their respective controls (Tables 1 and 2). The percentage decreases in weight gain were comparable in the two experiments. This effect is apparently linked to induced asparagine deficiency in each case, and cannot be accounted for by difference in feed intake in the nutritionally depleted group or in the asparaginase-treated group pair-fed with their controls. As the growth decrement was similar after chronic asparaginase treatment and after the feeding with asparagine-deficient diets, a requirement of asparagine for more efficient food utilization and optimal growth of weanling rats is suggested. This is consistent with low basal asparagine synthetase activity present in postnatal rat tissues (Patterson & Orr, 1969).

Determinations of organ wet weights showed small decreases in small-intestine and skeletal-muscle wet weight in both asparagine-depleted groups. After chronic (8-day) feeding of the asparagine-free diets or after the 8-day asparaginase treatment approx. 5 g decreases were found in the weights of decapitated whole bodies from which abdominal organs were removed, suggesting significant decreases in skeletal-muscle mass. Hence the significant lowering of asparagine concentrations in skeletal muscle and heart of adult female rats injected with growth hormone (Ottaway, 1972) and that reported here for skeletal muscle in all experimental groups (Tables 1 and 2) are of particular interest. However, the possibility that tissue glutamine deficiency also may have been induced in our dietary and enzyme-treated experimental groups should be considered, since glutamine is absent from the experimental free amino acid diets and the asparaginase preparation used may have some glutaminase activity.

We found that 3 h after a single injection of asparaginase a significant ($P < 0.05$) decrease in intracellular free asparagine concentration was found in kidney, spleen, liver and small intestines, and the decrease was even more pronounced in forebrain and skeletal muscle. Again, there were no significant effects on the free aspartate concentrations in any of

Table 1. *Effect of asparaginase treatment on the concentrations of asparagine and aspartate in tissues of prepubertal rats*

Group	Body-weight gain (%)	Free amino acid	Concn. in tissue ($\mu\text{mol/g wet wt.}$)				
			Brain	Small intestine	Spleen	Liver	Skeletal muscle
Control (saline injection)	65 $\pm$ 1.2	Asparagine	0.06 $\pm$ 0.01	0.22 $\pm$ 0.04	0.20 $\pm$ 0.01	0.29 $\pm$ 0.04	0.44 $\pm$ 0.01
Asparaginase injection 3 h before death	65 $\pm$ 1.2	Aspartate	2.20 $\pm$ 0.06	1.60 $\pm$ 0.11	3.80 $\pm$ 0.11	2.00 $\pm$ 0.14	0.44 $\pm$ 0.01
		Asparagine	0.00 $\pm$ 0.00*	0.13 $\pm$ 0.01*	0.07 $\pm$ 0.02*	0.10 $\pm$ 0.01*	0.03 $\pm$ 0.01*
Asparaginase-treated for 8 days	43 $\pm$ 3.8*	Aspartate	2.00 $\pm$ 0.11	1.10 $\pm$ 0.05	3.80 $\pm$ 0.19	1.30 $\pm$ 0.16	0.25 $\pm$ 0.08
		Asparagine	0.01 $\pm$ 0.00*	0.26 $\pm$ 0.00	0.23 $\pm$ 0.02	0.37 $\pm$ 0.05	0.27 $\pm$ 0.01*
		Aspartate	2.10 $\pm$ 0.12	1.30 $\pm$ 0.14	2.70 $\pm$ 0.35	1.80 $\pm$ 0.12	0.31 $\pm$ 0.08
							Kidney
							0.33 $\pm$ 0.01
							1.70 $\pm$ 0.27
							0.10 $\pm$ 0.04*
							1.80 $\pm$ 0.04
							0.30 $\pm$ 0.03
							1.70 $\pm$ 0.20

* Values statistically different ($P < 0.05$) from those of controls, as calculated by Student's *t* test.

Table 2. *Effect of the feeding (for 8 days) of an asparagine (and aspartate)-deficient diet on the concentrations of asparagine and aspartate in tissues of prepubertal rats*

See the legend to Table 1 and the text for experimental details. Each value represents the mean $\pm$ s.e.m. of eight replicate samples for each organ from four rats.

Dietary group	Body-weight gain (%)	Free amino acid	Concn. in tissue (μ mol/g wet wt.)					
			Brain	Small intestine	Spleen	Liver	Skeletal muscle	Testes
Control (complete amino acid mixture)	53 $\pm$ 0.7	Asparagine	0.06 $\pm$ 0.01	0.25 $\pm$ 0.02	0.34 $\pm$ 0.02	0.24 $\pm$ 0.05	0.40 $\pm$ 0.07	0.31 $\pm$ 0.07
		Aspartate	2.02 $\pm$ 0.05	0.87 $\pm$ 0.09	2.70 $\pm$ 0.22	2.30 $\pm$ 0.10	0.53 $\pm$ 0.07	1.30 $\pm$ 0.15
Asparagine-deficient (amino acid mixture deficient in asparagine and aspartate)	41 $\pm$ 1.8*	Asparagine	0.03 $\pm$ 0.01*	0.31 $\pm$ 0.04	0.28 $\pm$ 0.04	0.37 $\pm$ 0.05	0.15 $\pm$ 0.04*	0.27 $\pm$ 0.03
		Aspartate	1.96 $\pm$ 0.13	0.88 $\pm$ 0.17	2.70 $\pm$ 0.14	2.20 $\pm$ 0.08	0.53 $\pm$ 0.05	1.60 $\pm$ 0.15
								0.31 $\pm$ 0.09
								0.91 $\pm$ 0.07

* Values statistically different ($P < 0.05$) from those of controls, as calculated by Student's t test.

the tissues examined. In contrast with this effect of acute (3h) treatment with asparaginase, tissue asparagine concentrations were depressed significantly ($P < 0.05$) only in forebrain and skeletal muscle after the chronic (8-day) treatment with asparaginase. In animals maintained on diets free of asparagine (and glutamine and aspartate) the results were similar to those attained in the study of the effects of chronic treatment with asparaginase (Tables 1 and 2). There was a significant ($P < 0.05$) decrease in asparagine concentrations, but not those of aspartate, in forebrain and skeletal muscle; the decrease in the concentration of free asparagine in skeletal muscle was apparently even more pronounced after the chronic (8-day) nutritional depletion.

Hence one can propose that asparagine synthetase activity is not increased sufficiently 3h after an asparaginase administration, but that by 8 days the activity of asparagine synthetase apparently has helped to restore asparagine concentrations in all the tissues studied except in skeletal muscle and forebrain. The last-named tissues may be more dependent on plasma supplies of asparagine, and skeletal muscle may even function as a reservoir for asparagine (Christensen, 1964). The significant depletion of asparagine in plasma, brain, heart and skeletal muscle observed by Ottaway (1972) after the administration of growth hormone for 4-5 days is consistent with such a conclusion.

Hence it appears that short-term tissue asparagine depletion may affect protein synthesis, overall tissue growth and total body weight, particularly through decreases in total skeletal-muscle mass. The means by which asparagine depletion (and possibly glutamine depletion) could result in diminished general or specific protein synthesis leading to a retardation of tissue growth remains a substantially important question, particularly during the early growth and development of the rat.

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