Le NaF modifie aussi le profil enzymatique par l'apparition de l'isoenzyme c) dont la synthèse est fonction de la quantité de phosphates du milieu de culture. En présence d'une grande quantité de phosphates cette isoenzyme est absente mais apparait dans le cas d'une carence. Il est possible que le NaF en inhibant les activités phosphatasiques place le mycélium dans des conditions de carence en phosphates et entraîne la dérépression de l'isoenzyme c). La NaF, en empêchant les réactions enzymatiques pourrait laisser intact dans le mycélium un substrat non métabolisé capable alors de réprimer l'enzyme a).

Nous n'avons pas déterminé ni précisé le type de phosphatase acide qui serait soumis à une telle régulation. Mais HEREDIA[13] distingue chez les levures deux types de phosphomonoestérases à rôle physiologique différent, l'une étant répressible et dépendante du phosphate externe, l'autre constitutive interviendrait dans le métabolisme intermédiaire.

Summary. NaF inhibits the phosphatasic activities of mycelium *Saprolegnia monoïca* but brings about the repression of isozyme c, as does a phosphate deficiency. DL*p*FPA inhibits in a same manner the enzymatic phosphatasic activity and the protein synthesis.

M. FEVRE

Laboratoire de Physiologie végétale et Laboratoire de Mycologie associée au C.N.R.S. N°. 44, Université de Lyon I, 43, Boulevard du 11 Novembre 1918 F-69621 Villeurbanne (France), 4 Septembre 1973.

[13] C. F. HEREDIA, F. YEN et A. SOLS, Biochem. biophys. Res. Commun. *10*, 14 (1963).

[14] Je remercie Monsieur le Professeur G. TURIAN (Université de Genève) pour les critiques et suggestions fructueuses formulées au cours de la rédaction de ce manuscrit.

Effect of Low Protein Diet and Hyperammonemia on Liver Glutaminase Activity in the Rat[1]

Liver glutaminase effects release of ammonia from the amide group of glutamine for carbamyl phosphate action in the production of urea. As has been established for many other enzymes involved in amino acid catabolism, the activity of glutaminase decreases markedly during low protein intake[2].

Lack of glutaminase was once suggested to be the basic defect in lysinuric protein intolerance (LPI)[3–8]. This is an autosomal recessive disease[9] characterized by heavy lysinuria and amino nitrogen induced hyperammonemia. The patients have minimal protein intake because a strong aversion to protein is associated with the periodic hyperammonemia. Decreased liver glutaminase activity would be expected in LPI secondary to the restricted ingestion of protein, even if it was not the primary defect. In a previous work, the activity of glutaminase I, the major hepatic glutaminase, was determined in the liver of 2 LPI patients[10]. Surprisingly, an increased activity was found. Evidently some factor is involved, which tends to increase the glutaminase activity and exceeds the opposite effect of low protein intake. To assess the role of the periodic hyperammonemia, rats were kept on low protein diet and exposed intermittently to hyperammonemia, and the effect on liver glutaminase I activity was determined.

45 male Spraque-Dawley rats were divided into 4 experimental groups as shown in Table I. 2 of the groups received standard laboratory rat chow, with high quality protein 24.6% of the wet weight. The other 2 groups were placed on a low protein diet (Table II), containing 6% protein of the wet weight. Continuous free access to the diet and water was provided. The experiment lasted 14 or 21 days (Table I). One of the groups on each diet received i.p. injections of ammonium acetate 2 to 3 times daily for the 7 last days of the experiment. The single doses of ammonium acetate were 0.6 mmoles per kg in 0.1 *M* solution, which is 56% of the rat $LD_{99.99}$ and 73% of the LD_{50}[11]. The blood ammonia concentration, measured in 5 animals 1 h after the dose, ranged from 186 to 1090 μM (mean 427 μM), which is clearly above the upper limit of normal[12]. The animals were then decapitated, a segment

[1] This work was supported by The National Research Council for Medical Sciences, and the Foundation for Pediatric Research, Finland.

[2] J. R. BEATON, Can. J. Biochem. *41*, 1871 (1963).

[3] J. PERHEENTUPA and J. K. VISAKORPI, Lancet *2*, 813 (1965).

[4] M. KEKOMÄKI, J. K. VISAKORPI, J. PERHEENTUPA and L. SAXÉN, Acta paed. scand. *56*, 617 (1967).

[5] M. KEKOMÄKI, E. TOIVAKKA, V. HÄKKINEN and M. SALASPURO, Acta med. Scand. *183*, 357 (1968).

[6] M. KEKOMÄKI, N. C. R. RÄIHÄ and J. PERHEENTUPA, Acta paed. scand. *56*, 631 (1967).

[7] J. MALMQUIST and B. HETTER, Lancet *2*, 129 (1970).

[8] J. MALMQUIST, R. JAGENBURG and J. LINDSTEDT, New Engl. J. Med. *284*, 997 (1971).

[9] R. NORIO, J. PERHEENTUPA, M. KEKOMÄKI and J. K. VISAKORPI, Clin. Genet. *2*, 214 (1971).

[10] O. SIMELL, J. PERHEENTUPA and J. K. VISAKORPI, Pediat. Res. *6*, 797 (1972).

[11] J. P. GREENSTEIN, M. WINITZ, P. GULLINO and S. M. BIRNBAUM, Arch. Biochem. Biophys. *59*, 302 (1955).

[12] M. WINITZ, J. P. DE RUISSEAU, M. C. OTEY, S. M. BIRNBAUM and J. P. GREENSTEIN, Arch. Biochem. Biophys. *64*, 368 (1956).

Table I. Effect of low protein diet[a] and ammonium acetate treatment[b] on the growth of rats

	Treatment	Number of animals[a]	Weight at the beginning of the experiment mean ± SD (g)	Change in weight per day during experiment mean ± SD (g)
I	Normal diet	11 (3)	154.7 ± 10.8	3.39 ± 0.49
II	Normal diet and ammonium acetate	15 (8)	162.1 ± 14.8	1.48 ± 0.82
III	Low protein diet	7 (3)	156.7 ± 3.1	−0.08 ± 0.17
IV	Low protein diet and ammonium acetate	12 (8)	157.2 ± 10.0	−0.45 ± 0.31

[a] The experiment lasted 21 days, but some animals (numbers given in parentheses) received the diet for only 14 days. [b] Ammonium acetate was given i.p. for 7 days before the end of the experiment 2 to 3 times daily, a single dose being 0.6 mmoles per kg of ammonium acetate in 1.0 *M* solution.

reduced weight gain or caused a more pronounced drop in the weight. The average daily change in weight of the rats during the experiment is given in Table I.

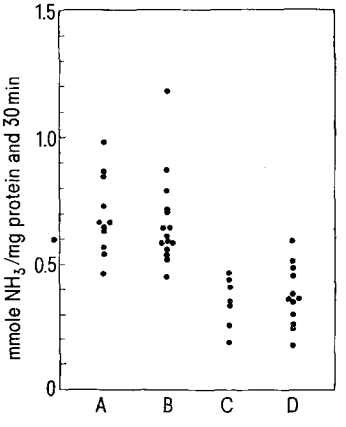
The glutaminase activity of the liver varied markedly in each group (Figure). The mean values (95% confidence of the liver was placed in ice-cold 0.9% saline and homogenized with a Potter-Elvehjem homogenizer, in 0.4 *M* phosphate buffer, pH 8.0, in an ice bath. Glutaminase I activity was measured with a modification of the method of SAYRE and ROBERTS[13]. 1.00 ml of 0.025 *M* L-glutamine in 0.4 *M* phosphate buffer was mixed with the homogenate and the final volume was adjusted to 2.00 ml with the buffer. The ammonia formed was measured in 0.2 ml samples at zero time and at 15 min intervals[14]; samples from simultaneous incubations without glutamine were used as blanks. Ammonia liberation proceeded linearly. The enzyme activity was expressed per protein content of the incubate[15].

The rats fed low protein diet ceased growing or lost some weight. Ammonium acetate treatment similarly

Table II. Composition of the low protein diet

Casein	1600.0 g
Sucrose (Ph. Nord.)	9600.0 g
Lard (Ph. Nord.)	7600.0 g
Corn oil (BP-63)	1000.0 g
Salt mixture [a]	999.8 g
α-tocopherol [b]	5.0 g
Calcium pantothenate [b]	1.0 g
Choline chloride [c]	140.0 g
Inositol puriss. [d]	7.0 g
Menadion [d]	0.1 g
Niacin pur. [c]	15.0 g
Pyridoxine chloride pur. [d]	0.5 g
Riboflavin pur. [d]	0.5 g
Thiamine chloride [b]	0.5 g
Vitol [e]	60.0 ml

[a] Salt mixture contained calcium carbonate 814.5, magnesium carbonate (Baker) 37.5, magnesium sulfate 24.0, sodium chloride 103.5, potassium chloride 168.0, potassium dihydrogen phosphate 318.0, ferric citrate. 5 H_2O (Merck) 68.2, potassium iodide 0.12, manganese sulfate 0.525, sodium fluoride 1.5, aluminum potassium sulfate 0.255, copper sulfate. 5 H_2O 1.35. [b] Roche. [c] Merck. [d] Fluka. [e] Orion Pharmaceutical Co, Helsinki: 1 ml contains 14,250 IU vitamin A, 10,000 IU calciferol.



Glutaminase I activity in the liver of rats fed normal (A, B) or low protein diet (C, D). B) and D) Ammonium acetate, 0.6 mmoles per kg in 1.0 *M* solution 2 to 3 times daily, was given i.p. for 7 days before the end of the experiment.

intervals) were 0.69 (0.54–0.84) for the normal diet group, 0.67 (0.43–0.85) for the normal diet + ammonium acetate group, 0.35 (0.15–0.55) for the low protein group and 0.38 (0.14–0.62) for the low protein + ammonium acetate group, in mmoles of ammonia liberated per mg protein in 30 min. The difference between the groups fed high or low protein diet was highly significant ($p < 0.0005$), but the ammonium acetate treatment was without effect on either diet group.

The marked drop in the activity of liver glutaminase in the rats on the low protein diet agrees with earlier findings[2]. In the kidney, ammonium chloride administration is followed by a rise in glutaminase I activity[16]. This is presumably a response to the acidosis and not to the hyperammonemia caused by ammonium chloride, since the liver glutaminase, at least, is unresponsive to ammonium acetate induced hyperammonemia according to the present data. Glutaminase I (phosphate dependent glutaminase), which has a far greater activity than glutaminase II (phosphate independent glutaminase) in the liver[17], is strongly activated by substrate excess and inhibited by the product, glutamate[18]. Many other factors, e.g. cold exposure of the animals[2], organic mercury compounds[19] and deficiency of vitamin B_6[20] influence activity of glutaminase through unknown mechanisms.

Patients with lysinuric protein intolerance live in continuous shortage of protein. The concentration of glycine, alanine, glutamate and glutamine as well as of ammonia is greatly increased in their plasma[4,8] and, presumably, also in their liver cells. Alanine is an important carrier of α-amino group in transamination reactions, and a large part of all metabolized amino nitrogen passes through it. The rat experiments suggest that the increase in liver glutaminase activity in LPI-patients is not a direct result of low protein diet and hyperammonemia. Possibly, it is a concomitant result of abnormal pooling and storage of amino acids in the pool glycine-alanine-glutamate-glutamine, increased neogenesis of these amino acids from ketoacids and recycling of transamination reactions to augment this stock, which is needed to balance the continuous deficiency of protein precursors and protein induced hyperammonemia in LPI-patients.

Zusammenfassung. Die Wirkung der periodischen Hyperammonämie auf die Glutaminase I in der Rattenleber wurde untersucht, wobei die Enzymaktivität während normaler und proteinarmer Ernährung mit und ohne i.p. Ammoniumazetat-Injektionen gemessen wurde. Während Ammoniumazetat in beiden Diätgruppen keinen Effekt auf die Aktivität hatte, war diese bei proteinarmer Diät auf die Hälfte der Norm herabgesetzt.

O. SIMELL

Children's Hospital, University of Helsinki, Stenbäckinkatu 11, SF–00290 Helsinki 29 (Finland),
16 August 1973.

[13] W. F. SAYRE and E. ROBERTS, J. biol. Chem. *233*, 1128 (1958).
[14] E.-L. RAHIALA and M. P. KEKOMÄKI, Clin. chim. Acta *30*, 761 (1970).
[15] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[16] L. GOLDSTEIN and C. J. KENSLER, J. biol. Chem. *235*, 1086 (1960).
[17] N. KATUNUMA, A. HUZINO and I. TOMINO, Adv. Enzym. Regul. *5*, 55 (1967).
[18] Z. KOVAĆEVIĆ, J. D. MCGIVAN and J. B. CHAPPEL, Biochem. J. *118*, 265 (1970).
[19] K. TAKAHAMA, Kumamoto med. J. *23*, 85 (1970).
[20] J. R. BEATON and M. E. GOODWIN, J. biol. Chem. *212*, 195 (1955).