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Glutamine synthetase activity in the ruminant spleen

The urea cycle amino acids[1] and the acidic amino acids, aspartic acid[2] and glutamic acid[3], when administered in a variety of ways, have been observed to lower mammalian blood ammonia levels. The mode of action of the two acidic amino acids is not thought to be a result of their ability to produce citric acid cycle intermediates. GREENSTEIN *et al.*[4] demonstrated that oxaloacetate or 2-oxoglutarate failed to prevent the toxicity to rats of a lethal dose of ammonium acetate. Glutamic acid could conceivably act by promoting the formulation of glutamine[5], presumably in some organ which contains glutamine synthetase (L-glutamate:ammonia ligase (ADP), EC 6.3.1.2) activity. High blood ammonia levels occur naturally in ruminants[6,7] and it is reasonable to assume that these animals are particularly endowed with an apparatus to deal with this potentially lethal situation.

A survey of the glutamine synthetase activity of a variety of organs from a wide range of animals, including several different mammals has been reported[8]. Among the organs tested were the spleens of sheep, pig, rabbit, mink and rat. It is interesting to note that the activity of the sheep spleen was very high while that of the other mammalian spleens tested was below the limit of this assay, at least 37 times lower than the activity recorded in sheep spleen. This difference was not observed in any other organ tested, values were presented, including at least one ruminant, for cerebrum, liver, cerebellum, heart, kidney, testis, lung and muscle.

The results with liver have been recently confirmed and extended to include goats, mouse, hamster, cat and vole[9]. It would appear that liver glutamine synthetase activity is a function of body size rather than nutrition. It was decided to extend this survey of spleen glutamine synthetase activities to include other ruminants.

Tissue samples were homogenized with a teflon glass homogenizer in ice-cold 0.1 M Tris–HCl (pH 7.2 at 37°) and used in the following incubation at 37° (final concentration): 8 mM ATP, 80 mM L-glutamate, 30 mM hydroxylamine, 30 mM L-cysteine, 150 mM Tris–HCl (pH 7.2) and 0.33% (w/v) homogenate. 3-ml samples of the incubation were withdrawn at intervals up to 24 min and added to 1 ml of a mixture of equal parts by volume of 10% $FeCl_3 \cdot 6\ H_2O$ in 2 M HCl, 6 M HCl and 24% (w/v) trichloroacetic acid. After bench centrifugation the extinction at 510 nm of the mixtures was determined. Results were computed assuming the ε_M of γ-hydroxamate of glutamate in the presence of acidic ferric chloride to be 520 (ref. 8).

Reaction rates were proportional to time under the conditions of routine assay, and initial reaction rates were proportional to homogenate concentration, at least up to 1.54%. Spleens were assayed immediately after killing the animal with the exception of those from goat which were stored for 2 weeks at −15° until required for assay. Storage of sheep spleen or rat liver at −15° for a similar period resulted in no loss of activity.

Results of the comparative survey can be seen in Table I and indicate that the ruminant spleen is unique in having a high glutamine synthetase activity.

Ruminants, owing to bacterial fermentation in the rumen, have high portal blood ammonia levels. Most of this ammonia is removed by the liver but this capacity can be exceeded both experimentally and naturally[6,7,10]. It would therefore not be

TABLE I

GLUTAMINE SYNTHETASE ACTIVITY IN MAMMALIAN SPLEEN HOMOGENATES

Incubations at 37° contained (final concentrations) 8 mM ATP, 80 mM L-glutamate, 30 mM hydroxylamine, 30 mM L-cysteine, 150 mM Tris–HCl (pH 7.2) and 3.3 mg/ml tissue. Glutamate γ-hydroxamate formation was determined by reaction with acidic ferric chloride. Results are expressed as mean ± S.E. Number of samples in parentheses. Lower limit of the assay system was 10 μmoles/g fresh tissue per h.

Animal	*Sex*	*Activity (μmoles γ-hydroxamate of glutamate formed per g fresh wt. of tissue per h)*
Guinea pig	Male	14 ± 5 (4)
Mouse	Male	< 10 (5)
Pig	Castrated male	< 10 (5)
Rat	Male	37 ± 3 (4)
Goat	Male and female	284 ± 63 (4)
Ox	Castrated male	277 ± 31 (4)
Sheep	Castrated male	210 ± 15 (4)

surprising for the ruminant to have a second defence against acute ammonia toxicity. A possible explanation for the high glutamine synthetase activity in this ruminant organ would be that it is a site of ammonia removal in these animals. GOLDSWORTHY *et al.*[11] recently studied the effect of arginine glutamate and aspartate on the utilization of ammonia by the perfused bovine liver. While they were able to demonstrate an increase in ammonia removal in the presence of aspartate and arginine, no increase could be demonstrated in the presence of glutamate. The authors conclude that the accelerated removal of ammonia by glutamate in the intact animals must take place elsewhere than in the liver. The results presented here indicate that an important site of ammonia removal may be the ruminant spleen.

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1 J. L. FAHEY, R. S. PERRY AND P. F. MCCOY, *Am. J. Physiol.*, 192 (1958) 311.
2 K. ABE, Y. KOWA, S. HARIGAYA AND T. MORITA, *Clin. Chem.*, 9 (1963) 475.
3 A. J. BARAK, F. L. HUMOLLER, D. J. MAHLER AND J. M. HOLTHAUS, *Gastroenterology*, 43 (1962) 35.
4 J. P. GREENSTEIN, M. WINITZ, P. GULLINO, S. M. BIRNBAUM AND M. C. OTEY, *Arch. Biochem. Biophys.*, 64 (1956) 342.
5 P. P. COHEN AND G. W. BROWN, *Comp. Biochem.*, 2 (1960) 161.
6 E. F. ANNISON, D. LEWIS AND D. B. LINDSAY, *J. Agr. Sci.*, 53 (1959) 34.
7 E. F. ANNISON, D. LEWIS AND D. B. LINDSAY, *J. Agr. Sci.*, 53 (1959) 42.
8 C. WU, *Comp. Biochem. Physiol.*, 8 (1963) 335.
9 E. V. ROWSELL, P. J. BUTTERY AND J. A. CARNIE. *Biochem. J.*, 115 (1969) 43P.
10 D. LEWIS, K. J. HILL AND E. F. ANNISON, *Biochem. J.*, 66 (1957) 587.
11 P. D. GOLDSWORTHY, M. D. MIDDLETON, K. A. KELLY, C. T. BOMBECK, T. AOKI AND L. M. NYHUS, *Arch. Biochem. Biophys.*, 128 (1968) 153.

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