The Effect of Protein Content of the Diet on the Rate of Urea Formation in Sheep Liver

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1. In the livers of six sheep given a high-protein diet, the concentrations of certain urea-cycle enzymes [ornithine transcarbamoylase, arginine synthetase (combined activity of argininosuccinate synthetase and argininosuccinase) and arginase] were significantly greater than when the sheep were given a low-protein diet. Alkaline phosphatase activity/mg. of liver protein was not significantly affected by diet. 2. Three sheep previously given the high-protein diet showed no significant rise in the concentration of ammonia in the blood after the administration of urea (0.5 g./kg. body wt.). The concentration of ammonia in the blood of the three sheep given the low-protein diet rose exponentially with time after dosing with urea and all sheep died. 3. It is suggested that tolerance to ammonia toxicity in the sheep is at least partly a function of the activity of the urea-cycle enzymes in the liver.

In simple-stomached ureotelic animals, ammonia entering the urea cycle is derived mainly from amino acids by deamination (Fruton & Simmonds, 1959). In ruminants, considerable quantities of ammonia are produced in the rumen by the micro-flora from amino acids derived from dietary protein and this ammonia may be absorbed by the portal system (McDonald, 1948). This suggests that, to cope with fluctuations in ammonia production due to variations in diet, the urea cycle could be of greater importance to ruminant than to non-ruminant animals.

Considerable quantities of urea are fed to ruminants as a source of nitrogen. During studies on the toxicity of urea and ammonium compounds to sheep (Morris & Payne, 1969), it was observed that the sheep's tolerance was markedly affected by the protein content of the diet. Since ingested urea is rapidly converted into ammonia by microbial activity (Lenkeit & Becker, 1938), and the toxicity of urea in the ruminant is due to the ammonia formed (Morris & Payne, 1969), the urea-cycle could well be implicated in the observed variations in tolerance to ingested urea. A number of reports have shown that the activities of arginase (reviewed by Ashida & Harper, 1961) and the enzymes of the urea cycle in general (Schimke, 1962) in rat liver are affected by the protein content of the diet.

The present paper reports the effects of two diets containing a high-protein and a low-protein content on the concentration of certain urea-cycle enzymes

in sheep liver and on the tolerance of these sheep to a toxic dose of urea.

MATERIALS AND METHODS

Experimental animals and rations. Six Merino × Border Leicester four-tooth wether sheep of about 60 kg. body wt. were taken from pasture, shorn, arranged in pairs on the basis of their body weight and housed in individual metabolism cages.

Two types of rations were given. A low-protein pellet was made from 60% milled wheaten straw, 25% low-protein sorghum grain, 8% corn flour, 5% molasses and 2% minerals + lower volatile fatty acids (Hemsley & Moir, 1963). A high-protein pellet was made from 60% lucerne chaff and 40% soya-bean-oil meal.

On an air-dry basis, the low-protein and the high-protein pellets contained 85% and 82% organic matter and 0.75% and 5.7% nitrogen respectively.

Experimental treatments. One member of each of the three pairs of wethers was fed daily for 30 days with 1 kg. of the low-protein pellet while the other member was fed daily with 1 kg. of the high-protein pellet. Samples of liver were taken from all six sheep and assayed for enzymes, and the feeding treatments were then reversed. After a further 36 days, samples of liver were again taken from all six sheep and assayed. The sheep were maintained on their respective rations for a further 4 days and then dosed via a stomach tube with 0.5 g. of urea/kg. body wt. in 1 l. of water. The changes in venous blood concentration of ammonia were measured at intervals up to 200 min. after urea administration. Surviving sheep were then slaughtered for the measurement of liver weight.

The activities of ornithine transcarbamoylase, arginine

synthetase and arginase were assayed to indicate the hepatic concentration of urea-cycle enzymes, and alkaline phosphatase activity was measured as an index of enzymes not concerned in urea synthesis. To demonstrate that differences between sheep in the concentrations of urea-cycle enzymes were correlated with the tolerances of the sheep to urea toxicity, sheep were dosed with urea and the changes in ammonia concentration of the blood were measured.

Preparation of liver homogenates. Samples of liver were obtained under local procaine anaesthesia by aspiration biopsy with the instruments described by Dick (1952). Immediately after their withdrawal, the cores of liver were placed in ice-cold Krebs-Ringer phosphate buffer (Umbreit, Burris & Stauffer, 1964) and within 30 min. were weighed and homogenized in a Potter-Elvehjem-type homogenizer with 19 vol. of water. Protein concentrations were determined on 1:50 dilutions of the liver homogenates by the method of Miller (1959).

Assay of liver enzymes. Ornithine transcarbamoylase (EC 2.1.3.3) was assayed on 1:50 dilutions of the homogenates by using the incubation conditions of Brown & Cohen (1959). The citrulline produced was determined by the method of Hunninghake & Grisolia (1966).

Undiluted liver homogenates were used for the assay of arginine synthetase [combined activity of argininosuccinate synthetase (EC 6.3.4.5) and argininosuccinase (EC 4.3.2.1)] by the incubation conditions of Brown & Cohen (1959). The urea produced was converted into ammonia by addition of urease during incubation and, after precipitation of protein, the resultant ammonia was determined by a modification of the method of Seligson & Seligson (1951).

Arginase (EC 3.5.3.1) was assayed on a 1:20 dilution of liver homogenate by the incubation conditions of Ashida & Harper (1961). The urea produced was determined by the method of Hunninghake & Grisolia (1966).

Alkaline phosphatase activity was assayed by the method of King & Armstrong as described by Carr (1953), with 0.2 ml. of undiluted liver homogenate in place of plasma.

Values for phosphatase were expressed in King-Armstrong units whereas the unit of activity of all other enzymes was μ moles of product formed/hr. at 38°.

Blood ammonia. Concentrations of ammonia in the blood were determined by a modification of the method of Seligson & Seligson (1951). Samples (2 ml.) of jugular blood were collected without stasis and anticoagulant and ejected into 2 ml. of ice-cold 20% (w/v) trichloroacetic acid in 15 ml. centrifuge tubes. After centrifugation, 1 ml. of the supernatant solution was used for determination of ammonia.

Statistical analyses. Results were analysed by analysis of variance according to Snedecor (1956).

RESULTS

The activities of ornithine transcarbamoylase, arginine synthetase, arginase and alkaline phosphatase in the livers of the sheep on the high-protein and the low-protein diets are presented in Table 1. The three enzymes concerned in urea synthesis were significantly affected by the diets, whereas the alkaline phosphatase activity/mg. of liver protein was unaffected. The low-protein diet resulted in a significant ($P < 0.03$) decline in the protein content of the liver, but this was insufficient to explain the decrease in the activities of the urea-cycle enzymes.

The weights of the livers from the six sheep at slaughter and the total activities of the four enzymes assayed are presented in Table 2. The protein content of the diet had a marked effect on the activities of the urea-cycle enzymes, but not on that of alkaline phosphatase. Although sheep given the low-protein ration had livers that weighed about 20% less than those of sheep given the high-protein diet, the body weights of the sheep given the two

Table 1. *Effects of high-protein and low-protein diets on the concentrations of sheep liver enzymes*

Animal no.	Protein content of diet	Liver protein (mg./g. wet wt.)	Ornithine transcarbamoylase activity		Arginine synthetase activity		Arginase activity		Alkaline phosphatase activity	
			(units/g. wet wt.)		(units/g. wet wt.)		(units/g. wet wt.)		(units/g. wet wt.)	
			(units/mg. of protein)	(units/mg. of protein)	(unit/mg. of protein)	(unit/mg. of protein)	(units/mg. of protein)	(units/mg. of protein)	(unit/mg. of protein)	(unit/mg. of protein)
1	High	159	9700	61	85	0.54	2980	18.7	2.8	0.017
	Low	160	8000	50	28	0.18	640	4.0	2.0	0.012
2	High	202	19400	96	116	0.57	1040	5.1	3.7	0.018
	Low	142	6100	43	54	0.38	420	2.9	2.7	0.019
3	High	187	11100	60	120	0.64	3040	16.2	4.0	0.021
	Low	162	4700	29	53	0.33	900	5.5	3.6	0.022
4	High	175	10900	62	108	0.62	2430	13.9	3.4	0.019
	Low	159	2900	19	49	0.31	960	6.0	2.1	0.013
5	High	198	9700	49	95	0.48	1380	7.0	3.4	0.017
	Low	162	4700	29	51	0.31	960	5.9	2.7	0.016
6	High	184	8200	45	98	0.53	2800	15.2	5.0	0.027
	Low	163	4900	31	53	0.33	1150	7.1	4.5	0.027
Mean	High	184.2	11500	62.0	103.7	0.56	2280	12.7	3.7	0.020
	Low	158.0	5230	33.3	48.0	0.31	840	5.2	2.9	0.018
Difference between means		$P < 0.03$	$P < 0.03$	$P < 0.02$	$P < 0.01$	$P < 0.01$	$P < 0.02$	$P < 0.04$	$P < 0.01$	Not significant

Table 2. *Effects of high-protein and low-protein diets on liver weight and total activities of enzymes*

Animal no.	Body wt. (kg.)	Protein content of diet	Liver wt. (g.)	$10^{-4} \times$ Ornithine transcarbamoylase activity (units)	$10^{-2} \times$ Arginine synthetase activity (units)	$10^{-4} \times$ Arginase activity (units)	$10^{-1} \times$ Alkaline phosphatase activity (units)
1	51.7	High	653	632	557	194	181
4	52.3	High	692	701	699	157	235
5	49.5	High	692	673	657	95	235
Mean							
$\pm$ s.d.	51.1		679 ± 62	669 ± 35	638 ± 67	149 ± 50	217 ± 31
2	49.2	Low	597	362	320	25	158
3	49.0	Low	505	238	267	45	182
6	42.7	Low	479	237	253	55	213
Mean							
$\pm$ s.d.	47.0		527 ± 25	279 ± 67	280 ± 35	42 ± 15	184 ± 26
Mean difference (% of value for sheep given high-protein diet)			22.3	58.3	54.8	71.8	15.2

Fig. 1. Changes in the concentration of ammonia in the blood of sheep after drenching with urea. Sheep on high-protein diet: $\square$, 1; $\circ$, 4; $\triangle$, 5; sheep on low-protein diet: $\blacksquare$, 2; $\blacktriangle$, 3; $\bullet$, 6.

diets just before challenge with urea were not markedly different (Table 2).

Sheep given the high-protein pellets almost invariably consumed their total daily ration, but sheep given the low-protein pellets left small residues that amounted to less than 20% of the feed offered to the sheep. Therefore the energy intakes of the sheep given the low-protein and the high-protein pellets would not be expected to differ

by more than about 20%. Owing to differences in rumen filling and to loss of fleece after shearing, both groups showed an apparent loss of body weight from their initial state.

Changes in the concentration of ammonia in the blood of the six sheep after they had been dosed with urea are presented in Fig. 1. For the three sheep given the low-protein pellet the concentration of ammonia in the blood increased exponentially with time until the sheep died. The clinical signs of these three sheep indicated that death was due to ammonia toxicity as described by Morris & Payne (1969). After dosage with urea, there was no significant increase in the concentration of ammonia in the blood of the three sheep previously given the high-protein pellets, and these sheep showed no clinical signs of ammonia toxicity.

DISCUSSION

Our results support the observations by Schimke (1962) that the protein content of the diet has a pronounced effect on the concentration of urea-cycle enzymes in the mammalian liver. However, Schimke (1962) demonstrated this effect of diet with rats given higher protein diets (15, 30 and 60%) than those used in our study. As there were no significant changes in the alkaline phosphatase activity in the liver/mg. of protein due to diet, it would appear that the protein content of the diet affects specific enzymes such as urea-cycle enzymes rather than the concentration of all enzymes in the liver. This observation is in agreement with the conclusion of Schimke (1963) that all conditions leading to protein breakdown are associated with increases in urea-cycle enzymes.

Schimke (1962) has shown from studies on isolated enzymes that the kinetic properties of urea-cycle enzymes from livers of rats given high-protein and low-protein diets are similar. This suggests that the enhanced activity of the enzymes in livers of sheep given high-protein diets is due to enzyme concentration.

The proportions of the activities of the three urea-cycle enzymes in sheep liver were of a similar order to those reported by Kennan & Cohen (1961) and Schimke (1962) for the livers of other mammalian species. The arginine synthetase and ornithine transcarbamoylase activities appear to be comparable or slightly lower in the livers of sheep. The observed values for arginase were lower than reported by the above workers, but activation with Mn^{2+} was not carried out, as Ashida & Harper (1961) have reported that activity after activation is proportional to initial activity.

These comparisons suggest that the urea cycle is probably not developed any further in the liver of the sheep than in other mammals.

Sheep given the high-protein diet had livers with a greater concentration of urea-cycle enzymes than sheep given the low-protein diet, and they also were more tolerant to ammonia toxicity from ingested urea. These observations provide strong presumptive evidence that the tolerance of sheep to ammonia toxicity is at least partly a function of activities of the urea-cycle enzymes in the liver.

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REFERENCES

- Ashida, K. & Harper, A. E. (1961). *Proc. Soc. exp. Biol., N.Y.*, **107**, 151.
Brown, G. W. & Cohen, P. P. (1959). *J. biol. Chem.* **234**, 1769.
Carr, J. J. (1953). In *Standard Methods of Clinical Chemistry*, vol. 1, p. 75. Ed. by Reiner, M. New York: Academic Press Inc.
Dick, A. T. (1952). *Aust. vet. J.* **28**, 234.
Fruton, J. S. & Simmonds, S. (1959). *General Biochemistry*, 2nd ed., p. 848. New York: John Wiley and Sons Inc.
Hemsley, J. A. & Moir, R. J. (1963). *Aust. J. agric. Res.* **14**, 509.
Hunninghake, D. & Grisolia, S. (1966). *Analyt. Biochem.* **16**, 200.
Kennan, A. L. & Cohen, P. P. (1961). *Proc. Soc. exp. Biol., N.Y.*, **106**, 170.
Lenkeit, W. & Becker, M. (1938). *Z. Tierernähr.* **1**, 97; cited by McDonald, I. W. (1948). *Biochem. J.* **42**, 584.
McDonald, I. W. (1948). *Biochem. J.* **42**, 584.
Miller, G. L. (1959). *Analyt. Chem.* **31**, 964.
Morris, J. G. & Payne, E. (1969). *J. agric. Sci.* (in the Press).
Schimke, R. T. (1962). *J. biol. Chem.* **237**, 459.
Schimke, R. T. (1963). *J. biol. Chem.* **238**, 1012.
Seligson, D. & Seligson, H. (1951). *J. Lab. clin. Med.* **38**, 324.
Snedecor, G. W. (1956). *Statistical Methods*, 5th ed., p. 237. Ames: The Iowa State College Press.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964). *Manometric Techniques*, 4th ed., p. 132. Minneapolis: Burgess Publishing Co.