can be drawn at the present time. Further studies are now in progress using a larger number of carbohydrates of different solubility to determine whether such findings hold true in every instance.

References

1. ARNOLD, F. A. *Publ. Hlth. Rep.*, Wash., 1942, **57**, 1599.
2. COX, G. J. *et al.* *J. dent. Res.*, 1948, **27**, 562.
3. KEYES, P. H. *J. dent. Res.*, 1944, **23**, 439.
4. *Ibid.*, 1946, **25**, 341.
5. KEYES, P. H. and DALE, P. P. *J. dent. Res.*, 1944, **23**, 427.
6. MCCLURE, F. J. *J. dent. Res.*, 1945, **24**, 239.
7. SCHWEIGERT, B. S. *et al.* *J. Nutrition*, 1945, **29**, 405.
8. SHIBATA, M. *J. exp. Med.*, Japan, 1929, **7**, 247.

Synthesis of Amino Acids in the Rumen

J. K. Loosli, H. H. Williams, W. E. Thomas, Fent H. Ferris, and L. A. Maynard

Departments of Animal Husbandry and Biochemistry, and Nutrition, Cornell University, Ithaca, New York

In 1891 Zuntz (*6*) presented the view that bacteria in the rumen of animals utilize nonprotein nitrogenous compounds to form protein, which in turn was used by the animals. In recent years it has been conclusively shown that protein is formed in the rumen from dietary urea and ammonium salts. The protein thus formed appears to be of relatively low biological value (*3–5*) but, to our knowledge, no attempt has been made to measure the amino acid composition of protein synthesized in the rumen.

In the course of studies designed to determine the amino acid requirements of farm animals, samples of diets, rumen material, and excreta obtained from three sheep and two goats fed a purified diet containing urea as the nitrogen source were analyzed for the ten essential amino acids. The animals were fed a diet containing corn sugar, 25%; cornstarch, 42%; cellophane, 20%; minerals, 5%; lard, 4%; and urea, 4%. Vitamins A and D were fed separately each day, but the B vitamins were not supplied because most of them have been shown to be synthesized in ruminants. After the animals had been on a constant amount of the diet for at least 20 days, collections of urine and feces were made for a 10-day period. Samples of rumen material were obtained by stomach tube at the end of the collection periods.

Lambs fed the urea-containing diet gained an average of 0.23 lb per day, as compared with 0.30 lb for similar lambs fed concurrently a ration containing casein. All animals were in positive nitrogen balance. As an average they stored 1.18 g of nitrogen each day. Biological values, calculated from the nitrogen balance data obtained from these animals and figures reported in the literature (*2*) for metabolic and endogenous nitrogen gave values of 56 for the diet containing urea and 82 for the casein ration.

The amino acids were determined in the purified diet, rumen material, and excreta by the microbiological technique after hydrolysis.

Hydrolysis was carried out in the autoclave in sealed tubes. Acid (10% HCl) was used for all amino acids except tryptophan. Alkali (5 N NaOH) was used for the latter. Organisms, obtained from the American Type Culture Collection, Georgetown University, Washington, D. C., were used for assay of the different amino acids as follows: *Streptococcus faecalis* #9790 for arginine, threonine, tryptophan, and valine; *Leuconostoc mesenteroides* P-60 #8042 for histidine, lysine, methionine, and phenylalanine; and *Lactobacillus arabinosus* 17–5 #8014 for isoleucine and leucine. The basal medium was the same as that reported in the literature for each organism, with the exception of slight modifications to be reported later (*1*).

In the analysis of rumen material, inhibition of growth of microorganisms was noted at the higher assay levels. This is being investigated further. The values for synthesis of amino acids in the rumen therefore are minimal.

TABLE 1

AMINO ACID CONTENTS OF RUMEN, FECES, AND URINE SAMPLES; AND DAILY AMINO ACID BALANCE OF SHEEP AND GOATS FED UREA DIET

Amino acid	Amino acid content (g/16 g N)				Apparent daily amino acid in g				
	Diet	Rumen material	Feces	Urine	Intake from		Losses in		Retention
					Diet	Rumen*	Feces	Urine	
Arginine	0.47	3.09	3.43	0.32	0.19	1.27	0.48	0.06	0.73
Histidine	0.13	1.44	1.27	0.12	0.05	0.59	0.18	0.02	0.39
Isoleucine	0.00	3.38	3.72	0.31	0.00	1.38	0.52	0.06	0.80
Leucine	0.36	4.96	4.35	0.43	0.15	2.04	0.61	0.08	1.35
Lysine	0.63	5.71	5.09	0.61	0.24	2.34	0.71	0.12	1.51
Methionine	0.08	1.62	1.48	0.09	0.03	0.66	0.21	0.02	0.43
Phenylalanine	0.13	2.47	3.39	0.22	0.05	1.01	0.48	0.04	0.49
Threonine	0.16	3.98	4.77	0.32	0.07	1.63	0.67	0.06	0.90
Tryptophan	0.04	0.61	0.94	0.04	0.01	0.25	0.13	0.01	0.11
Valine	0.34	3.82	4.89	0.38	0.14	1.57	0.69	0.08	0.80

* These values were calculated by multiplying the daily nitrogen intakes by the amino acid contents of the rumen material.

On the basis of the assays, the purified diet contained traces of all amino acids studied except isoleucine, even though urea was the only nitrogen source added. The presence of these amino acids in the purified diet appears to be a slight protein contamination from the cornstarch, sugar, and lard. The rumen material contained 9 to 20 times more of the amino acids that the diet fed (Table 1).

From a calculation of the amounts of amino acids furnished by the daily ration it would appear that the losses in feces and urine considerably exceeded the dietary intake. Obviously, this could not be the case because the animals were storing nitrogen and gaining weight. If the amino acid content of the rumen material is used to estimate the amounts available to the animals, a retention of the amino acids is indicated, which would explain the ability of the animals to grow. The fact that the animals have continued to gain in weight on the urea diet, containing no protein, for over 3 months is further evidence of the formation of amino acids.

Similar studies were carried out using a purified diet containing glycine as the only source of nitrogen. Rumen samples from sheep fed the glycine diet again indicated synthesis of the amino acids but at a lower level.

Results of these experiments show that the ten essential amino acids are synthesized in large amounts in ruminants fed urea as the only dietary source of nitrogen.

(Supported in part by a grant from the Frausch Foundation.)

References

1. BRIM, MYRON. *The evaluation of some lactic acid producing bacteria in the microbiological assay of amino acids.* Ithaca, New York: Cornell University, 1948.
2. HARRIS, L. E. and MITCHELL, H. H. *J. Nutrition,* 1941, **22**, 167; 183.
3. LOFGREEN, G. P., LOOSLI, J. K., and MAYNARD, L. A. *J. animal Sci.,* 1947, **3**, 343.
4. LOOSLI, J. K. and HARRIS, L. E. *J. animal Sci.,* 1945. **4**, 435.
5. RUPEL, I. W., BOHSTEDT, G., and HART, E. B. *J. dairy Sci.,* 1943, **26**, 647.
6. ZUNTZ, N. *Pflug. Arch. f. Physiol.,* 1891, **49**, 477.

Experimental Amyloidosis in the Guinea Pig[1]

C. L. Pirani, C. G. Bly, K. Sutherland, and F. Chereso

Army Medical Nutrition Laboratory, Chicago* and *Department of Pathology, College of Medicine, University of Illinois

In the course of a pathological study of chronic scurvy, we unexpectedly observed deposits of amyloid in various organs. So far as we know, this is the first report of experimental production of amyloidosis in guinea pigs.

Thirty young guinea pigs were fed scorbutogenic diets for varying lengths of time as shown in Table 1; 12 received the Sherman-La Mar diet as modified by Rinehart (*10*), and 18 the Crampton No. 5 diet (*3*). The control animals received 2.0 mg/day ascorbic acid, the acute scurvy group none, and the chronic scurvy group 0.2 mg/day. No amyloid was found either in the control or in the acute scurvy animals. Among the chronic scurvy guinea pigs, amyloid was demonstrated only in those animals which were sacrificed 8 weeks or longer after the beginning of the experiment. Six out of seven animals in this last group showed distinct amyloidosis. The lone exception was that of a guinea pig on the Crampton No. 5 diet, which was sacrificed at 19 weeks following a period of 4 weeks of unexplained clinical improvement.

TABLE 1

EFFECT OF DIET AND ASCORBIC ACID ON AMYLOID DEPOSITION IN GUINEA PIGS

Diet	Ascorbic acid supplement (mg/day)	Number of animals	Time sacrificed (weeks)	Pathological findings		
				Inanition	Scurvy	Amyloid
Rinehart	0	4	4–5	0	acute	0
	0.2	5	9–18	+	chronic	+ (in all 5)
	2.0	3	1–18	0	0	0
Crampton No. 5	0	7	4–5	0	acute	0
	0.2	2	8 and 14	+	chronic	+ (in both)
	0.2	5	1, 1, 2, 6, 19	+ (6–19 weeks)	+ (6–19 weeks)	0
	2.0	4	8–17	0	0	0

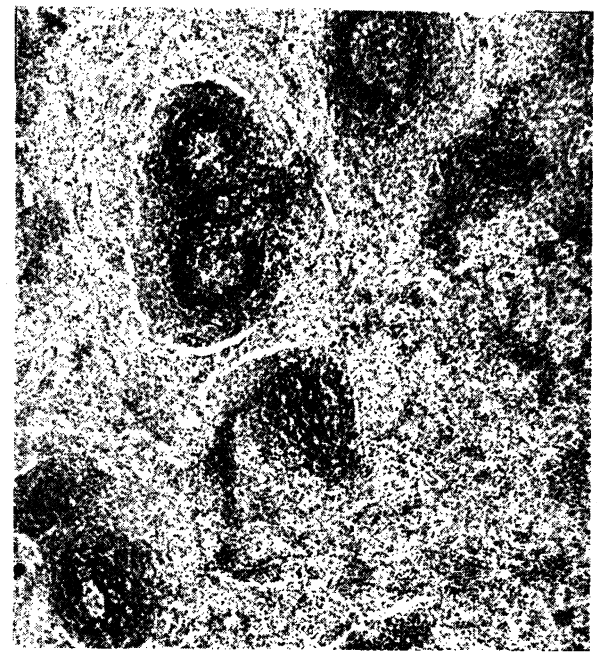

FIG. 1. Spleen from a control guinea pig (hematoxylin-eosin; × 65). Army Institute of Pathology.

[1] The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any governmental agency.