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The formation of urea in autolysis.

V. C. MYERS, M. RINGER and O. O. BENSON, JR.

[*From the Biochemical Laboratories of the New York Post-Graduate Medical School and Hospital, New York City, and the State University of Iowa, Iowa City, Iowa.*]

As a result of the recent experiments of Bollman, Mann and Magath[1] there is now little doubt that the liver plays the leading rôle in urea formation. Nevertheless very little is known concerning the mechanism of urea formation. To be sure we have long known from the experiments of Kossel and Dakin[2] that arginine may be split dircetly into urea and ornithine under the action of a specific enzyme, arginase, but relatively this accounts for only a small amount of the urea formed.

We wish to record observations of autolysis experiments carried out some years ago[3] (1914) which we have recently confirmed and extended[4]. The original motive for initiating these experiments was the availability of a specific method for estimating urea (Marshall). We were able to show then that whereas urea formation took place in autolyzing liver tissue, no change in the concentration of urea occurred in muscle tissue similarly treated.

Since our first experiments were carried out somewhat simi-

[1] Bollman, J. L., Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1924, lxix, 371.

[2] Kossel, A., and Dakin, H. D., *Z. physiol. Chem.*, 1904, xli, 321.

[3] Ringer, M., Experiments toward an ultimate determination of the site of urea formation. Thesis, C. C. N. Y., 1915.

[4] Benson, Jr., O. O., The formation of urea in the animal organism, Master's Thesis, State University of Iowa, 1925.

lar results have been obtained by several workers. Hoagland and Mansfield,[5] contrary to their expectations, obtained entirely negative results in muscle autolyses. Fosse and Rouchelman,[6] employing the xanthydrol method, were able to demonstrate a six fold increase of urea in autolyzing liver tissue, a change which was entirely inhibited by boiling the liver. Fosse[7] believes that the intermediate substance in urea formation is cyanic acid. McCance[8] has endeavored to ascertain the optimum conditions for urea formation in autolyses. He reports urea formation not only in liver but also in spleen.

Data illustrating the results which were obtained in our earlier experiments are given in Table 1. As will be noted there is no

TABLE I. Muscle and Liver Autolyses (1914).

Tissue employed	Time autolyzed at 38° C.	Urea N	Ammonia N
	days	mg. per 100 gm.	mg. per 100 gm.
	0	21.4	13.8
	4	22.4	22.4
Muscle	12	21.4	25.0
Cat 17	16	23.3	26.1
	20	23.6	27.5
	28	25.7	28.6
	56	22.4	36.8
	0	7.0	23.3
Liver	8	31.1	43.5
Cat 18	14	87.1	74.6
	21	128.1	155.5

evidence of any urea formation in the muscle autolyses, but in the liver autolyses concentration of the urea increased more than ten fold.

In addition to estimations of urea and ammonia nitrogen our recent analyses have included estimations of amino acid nitrogen as well. A few of our observations are given in Table II. These do little more than confirm our earlier experiments. The slight rise in amino acid nitrogen does not appear to have any special significance. It will be noted that in the liver autolyses there was a 300 mg. increase in urea nitrogen in about five days. In the mixed muscle-liver autolyses there was a considerable in-

[5] Hoagland, R., and Mansfield, C. M., *J. Biol. Chem*, 1917, xxxi, 487.

[6] Fosse, R., and Rouchelman, N., *Comp. rend. Acad. Sci.*, 1921, clxxii, 771.

[7] Fosse, R., *Bull. Soc. chim. biol.*, 1920, ii, 4.

[8] McCance, R. A., *Biochem. J.*, 1924, xviii, 487; 1925, xix, 134.

TABLE II. Muscle and Liver Autolyses (1925).

Tissue employed	Time autolyzed at 38° C.	Urea N	Ammonia N	Amino acid N
		mg. per 100 gm. of tissue		
	hours			
	1	35.7	11.7	46.4
Rabbit muscle	12	34.8	18.6	46.6
Exp. 7	24	36.2	21.3	49.3
	48	34.3	22.1	57.2
	96	36.7	42.1	58.1
	2	46.7	27.7	46.0
	10	182.0	49.0	49.3
Calves' liver	20	319.5	55.2	50.5
Exp. 10	44	321.0	65.2	53.4
	88	325.0	67.1	55.5
	136	341.0	77.1	—
Mixture of rabbit	0	34.0	25.4	45.9
muscle and liver	12	37.6	35.8	49.4
17 per cent liver	36	76.8	50.0	50.5
Exp. 13	84	94.9	60.1	54.4

crease in urea, but the amount was no greater than could be explained on the basis of the liver tissue alone.

There is no doubt that urea originates in the autolyses of liver tissue. Liver perfusion experiments have clearly shown that ammonium salts and some amino acids, but not all, give rise to urea.[9] These substances are probably not the immediate precursors of urea and it would seem likely that there was an intermediate stage in urea formation. We believe that suitably planned autolyses experiments with liver tissue and extracts should disclose the immediate precursor of urea, although efforts in this direction have thus far been negative.

[9] Löffler, W., *Biochem. Z.*, 1916, lxxvi, 55.