PN 1257

On the metabolism of ε-*N*-methyl-L-lysine by rat-kidney homogenate

It has been reported that ε-*N*-methyl-DL-lysine can support the normal growth of rats feeding on a lysine-deficient diet[1]. ε-*N*-Methyl-DL-lysine has also been shown to be metabolized by rat-kidney slices. These same authors were able to demonstrate the release of free lysine as well as the simultaneous consumption of oxygen, but were unable to detect the formation of formaldehyde. However, when the ε-*N*-methyl-DL-lysine was incubated with rat kidney which has been homogenized in phosphate or bicarbonate buffer, no evidence that it was metabolized could be obtained.

In the work reported here, we have shown that when rat kidney is homogenized in water or isotonic sucrose solution, and the homogenate is subsequently incubated with ε-*N*-methyl-D-lysine in a buffer of very low ionic strength, there is an oxygen consumption, a release of free lysine and a formation of formaldehyde, all in stoichiometric amounts. Furthermore, we have shown that the enzyme or enzyme system in rat kidney, which is responsible for the metabolism of ε-*N*-methyl-L-lysine, is located in the mitochondria, and that it is definitely different from "demethylase"[2], and from sarcosine oxidase.

α-*N*-Methyl-L-lysine and ε-*N*-methyl-L-lysine were synthesized by methylation of the appropriate *N*-*p*-toluenesulfonyl-*N*-benzoyl-L-lysine followed by hydrobromic acid hydrolysis (details will be published elsewhere). Sarcosine was purchased from Nutritional Biochemical Company, *N*-methyl-L-leucine from K & K Laboratories, Inc., *N*-methyl-DL-valine from Sigma Chemical Company, and the lysine decarboxylase preparation, an acetone powder of *Bacterium cadavaris*, from Worthington Biochemical Company.

Kidneys from a 200-g Sprague–Dawley rat were homogenized in 4 vol. of water or 0.25 M sucrose solution using a glass homogenizer. An incubation mixture was prepared containing 2.5 ml of homogenate, 1.0 ml of 0.08 M phosphate buffer (pH 7.0), 1.0 ml of an aqueous solution containing 50 μmoles of ε-*N*-methyl-L-lysine, and 0.5 ml of water or other solution to be tested. The mixture was incubated for 1 h at 37° and then boiled for 2 min to terminate the reaction. Oxygen consumption was recorded by Warburg manometer.

Formaldehyde formed was trapped with semicarbazide and measured as described[3]. Lysine liberated was determined on an aliquot of the supernatant by the use of lysine decarboxylase of *Bacterium cadavaris* as previously described[4]. Protein was determined by the method of LOWRY *et al.*[5]. Mitochondria were prepared as described by SCHNEIDER[6]. The activity of the enzyme is expressed either as μmoles of substrate metabolized per h per mg of protein, or as μl of CO_2 evolved per h per mg of protein. 19.9 μl of CO_2 corresponded to 1 μmole of L-lysine liberated.

Fig. 1 shows that the metabolism of ε-*N*-methyl-L-lysine by rat-kidney homogenate is very much dependent on the ionic strength of the medium. It also shows that the nature of the ions present also exerts a great influence on the enzymatic activity. It is therefore not impossible that the reason why NEUBERGER AND SANGER[1] failed to obtain positive results with a homogenate is that they were using a buffer of too high strength.

Table I gives the stoichiometry between the amount of formaldehyde formed, the oxygen consumed, and the L-lysine liberated from the ε-*N*-methyl-L-lysine. The

failure of NEUBERGER AND SANGER[1], to detect the formation of formaldehyde in their experiments could have been due to its rapid utilization by rat kidney[7].

In Table II are listed ε-*N*-methyl-L-lysine and several other *N*-methyl amino acids which were tested for susceptibility to oxidation by different enzyme preparations. It is seen that the oxidation of ε-*N*-methyl-L-lysine occurred mainly in the kidney, while that of sarcosine occurred mainly in the liver. It is generally agreed that the sarcosine oxidase in rat is found mostly in the liver[8]. Furthermore, the table

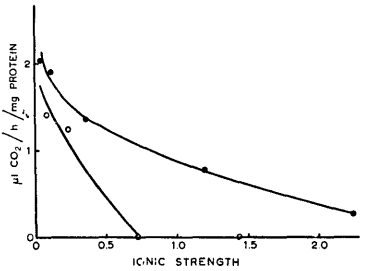

Fig. 1. Effect of ionic strength and species of ions on the metabolism of ε-*N*-methyl-L-lysine by rat homogenate. The incubation was carried out at pH 7.0. ●—●, in phosphate buffer; O—O, in KCl.

TABLE I

STOICHIOMETRY OF FORMALDEHYDE FOUND, OXYGEN CONSUMED, AND LYSINE LIBERATED FROM ε-*N*-METHYL-L-LYSINE BY RAT KIDNEY HOMOGENATE

	Formaldehyde (μmoles) *formed*	L-*Lysine* (μmoles) *liberated*	*Oxygen* (μmoles) *consumed*
1*	7.59	8.34	
2		7.38	7.45

* Incubation in the presence of 13.8 μmoles of semicarbazide. A separate incubation was carried out for the lysine determination.

TABLE II

OXIDATION OF A FEW *N*-METHYL AMINO ACIDS BY RAT-KIDNEY HOMOGENATE AND MITOCHONDRIA PREPARATION

	Homogenate		*Kidney mitochondria*
	Liver	*Kidney*	
ε-*N*-Methyl-L-lysine	0	0.395*	0.933
α-*N*-Methyl-L-lysine	0	0	0
N-Methyl-DL-valine	0	0	0
N-Methyl-L-leucine	0	0	0
Sarcosine	0.209	0.107	0.124

* The activity is expressed as μmoles substrate oxidized per h per mg protein. Total volume of incubation mixture in Warburg vessel is 3 ml, thus, two-thirds of the volumes of the reagents mentioned in methods were introduced. Center well contained 0.2 ml of 20% KOH.

shows that whereas ε-*N*-methyl-L-lysine is metabolized by kidney mitochondria at more than twice the rate for kidney homogenate, sarcosine is oxidized at roughly the same rate by both preparations. It is therefore unlikely that the enzyme responsible for the oxidation of ε-*N*-methyl-L-lysine is sarcosin oxidase.

It is also seen in Table II that the other four α-*N*-methyl amino acids tested were not at all oxidized by liver or kidney homogenate, or even kidney mitochondria. The fact that ε-*N*-methyl-L-lysine was oxidized while these four α-*N*-methyl amino acids were unaffected indicates that the enzyme responsible for the oxidation of the former is not "demethylase" as this enzyme is known to oxidize the α-*N*-methyl amino acids tested. It is also pertinent to add that "demethylase" has been shown to be a "soluble" enzyme[7].

Even though identification of the enzyme system responsible for the oxidation of ε-*N*-methyl-L-lysine must await its purification, the results cited suggest that an enzyme system not known up to date is involved. Purification of the enzyme is presently under progress.

Regarding the reaction mechanism for the enzymatic demethylation of ε-*N*-methyl-L-lysine, it is quite possible that it is similar to that of demethylation by "demethylase"[2].

This work was supported by research grants (A-6094 PC) from the Institute of Arthritis and Metabolic Disease, U.S. Public Health Service and from the Medical Research Council of Canada.

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Received February 14th, 1963