TABLE II

THIAMINE SYNTHESIS FROM OMP- AND TH-PHOSPHATE ESTERS WITHOUT ATP

The complete system contained 200 μmoles Tris buffer (pH 7.0), 10 μmoles cysteine, 0.1 μmole OMP-P or OMP-PP, 0.1 μmole Th, Th-P or Th-PP and 5 mg protein.

Substrates	*Thiamine formed mμmole 2 h*
OMP-P + Th-P	1.4
OMP-P + Th-PP	0.4
OMP-P + Th	0
OMP-PP + Th	1.7
OMP-PP + Th-P	63.2
OMP-PP + Th-PP	22.2
OMP* + Th	11.3

* Original complete system containing 10 μmoles ATP and 10 μmoles $MgCl_2$.

thiamine formed consists of free and phosphorylated thiamine and the enzyme system contains phosphatase activities.

The authors wish to thank Dr. A. FUJITA for his advice and encouragement and also Dr. F. LIPMANN for his kind revision, and to acknowledge the support of a grant from Scientific Research of Education Ministry.

YOSHITSUGU NOSE
KIYOSHI UEDA
TAKASHI KAWASAKI

Biochemical Institute, Kyoto Prefectural University, School of Medicine, Kyoto (Japan)

1 D. W. WOOLLEY, *Nature*, 171 (1953) 323.
2 D. L. HARRIS AND J. YAVIT, *Federation Proc.*, 16 (1957) 192.
3 M. E. JONES AND F. LIPMANN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, Inc., New York, 1955, p. 585.
4 A. FUJITA, Y. NOSE, S. KOZUKA, T. TASHIRO, K. UEDA AND S. SAKAMOTO, *J. Biol. Chem.*, 196 (1952) 289.
5 J. WEIJLARD, *J. Am. Chem. Soc.*, 64 (1942) 2279.

Received April 28th, 1959

On the biosynthesis of felinine

DATTA AND HARRIS[1] noted the existence of a new ninhydrin-positive spot upon paper chromatography of cat urine. WESTALL[2] isolated this material, which he called felinine, and obtained evidence indicating that its structure was that of S-(3-hydroxy-1:1-dimethylpropyl)cysteine

$$HOOC\text{-}CH(NH_2)CH_2\text{-}S\text{-}C(CH_3)_2\text{-}CH_2\ CH_2OH$$

This structural assignment was confirmed by synthesis by TRIPPETT[3].

We have developed an analytical method for felinine based on an ion-exchange chromatographic separation, followed by development of the ninhydrin color in the appropriate fractions, and have obtained evidence that cystine on the one hand, and either leucine or mevalonic acid on the other, can contribute to the formation

Abbreviation: MVA, mevalonic acid.

of this amino acid in the cat. The adult cat excretes more than 175 mg felinine/100 ml urine, whereas none of the other amino acids, with the exception of creatinine, are present at levels in excess of 6 mg/100 ml[4]. For this reason, application of a 0.2-ml aliquot of urine, purified by a slight modification of the procedure of WESTALL[2], to an Amberlite column in the acid form (0.9 × 20 cm, packed with Amberlite IR 120, Type III, particles 22–35 μ in diameter, conditioned and sized by the method of HAMILTON[5]) followed by elution with HCl (120 ml 0.37 *N* HCl, then 60 ml 1.1 *N* HCl, then 1.7 *N* HCl; collected 1-ml fractions, flow rate 30 ml/h) leads to the appearance of only 2 major ninhydrin-positive peaks. The first, which corresponds to felinine (established by comparing both on the column and by paper chromatography in two solvent systems with a sample of natural felinine) appears at approx. 140 ml, and the second, corresponding to creatinine, at 160–165 ml.

With the help of this analytical method, it was possible to show, as may be seen in Fig. 1, that the administration of cystine, leucine, or MVA to the cat markedly elevated urinary felinine excretion.

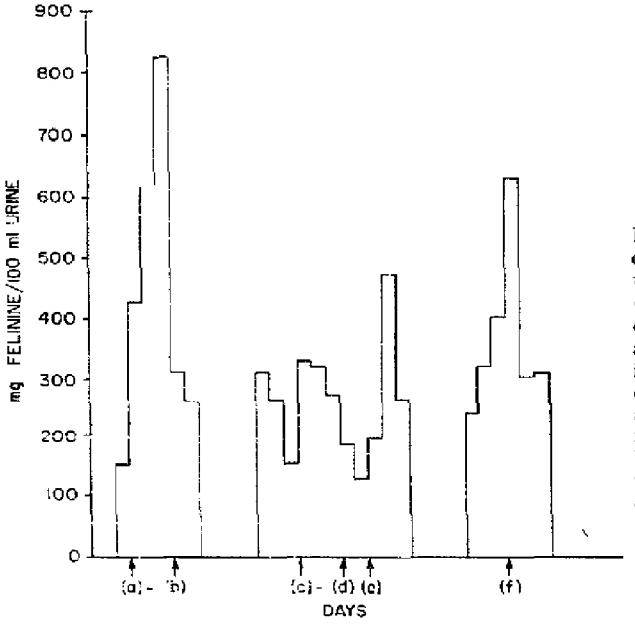

Fig. 1. Effect of administration of L-cystine, L-leucine and DL-MVA on the urinary excretion of felinine in the cat. (a)–(b): 1 % L-cystine incorporated in the diet for 3 successive days starting at (a) and stopping at (b). (c)–(d): 1 % L-leucine incorporated in the diet for 3 successive days starting at (c) and stopping at (d); at (e), a total of 1.5 g L-leucine dissolved in the minimum volume of phosphate buffer (0.1 *M*, pH 7.4) injected intraperitoneally in 3 doses of approx. 50 ml each. (f): Approx. 1.5 g DL-MVA (prepared from the DBED salt immediately prior to use) dissolved in phosphate buffer, injected intraperitoneally.

We have obtained confirmatory evidence for the participation of leucine and MVA in felinine biosynthesis by utilizing ^{14}C-labeled compounds. Table I shows that significant activity is found in felinine when either DL-[2-^{14}C]leucine or DL-[2-^{14}C]-MVA are injected intraperitoneally into the cat. In the radioleucine experiment, felinine was the only compound in the urine containing significant radioactivity. With DL-[2-^{14}C]MVA, however, by far the greater share of the activity in the urine was eluted from the ion-exchange column in the first fraction (0–20 ml). This material did not give a ninhydrin-reaction, was found to behave in the same way as a synthetic sample of DL-[2-^{14}C]MVA upon paper chromatography and is assumed to be the unnatural form of [2-^{14}C]MVA. That felinine is responsible for the radioactivity in the

felinine fraction from the ion-exchange column in both experiments, and not traces of highly active material, was also established by paper chromatography.

TABLE I

UTILIZATION OF DL-[2-^{14}C]LEUCINE AND DL-[2-^{14}C]MVA FOR FELININE BIOSYNTHESIS IN THE CAT

Compound injected	*Total activity (counts/min) in:*		
	Radioactive precursor	*Isolated compound*	
DL-[2-^{14}C]Leucine*	779,000	Felinine	16,500
DL-[2-^{14}C]Mevalonic acid**	970,000	MVA	408,000
		Felinine	5,100
		Creatinine***	3,000

* 15 mg (specific activity, 0.05 mC/mmole) diluted to 200 mg with unlabeled DL-Leucine and recrystallized. Injected 100 mg.

** 14 mg (as DBEI[1] salt, specific activity 0.05 mC/mmole) converted to MVA and diluted to 140 mg with unlabeled DL-MVA. Injected 120 mg.

*** Identified by comparison of the elution position from the ion-exchange column, and the R_F value upon paper chromatography in *tert.* butanol–acetic acid–water, with the values for an authentic sample of creatinine.

Investigations bearing on the mechanism of biosynthesis of felinine, and upon the possibility that felinine biosynthesis plays a regulatory role in the metabolism of MVA or leucine, are under way in this laboratory.

It has recently come to our attention that Drs. SIMON BLACK and N. BAUMAN, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md., are also engaged in studying the biosynthesis of felinine. In unpublished work, they have found that [^{14}C]acetate and DL-[2-^{14}C]MVA can serve as precursors of radiofelinine.

The authors would like to thank Dr. JAMES H. SPRAGUE and Dr. KARL FOLKERS of the Merck Sharp and Dohme Laboratories for generous supplies both of nonradioactive and ^{14}C-labeled MVA, and Dr. R. G. WESTALL of the University College Hospital Medical School, London, for a sample of natural felinine.

Department of Chemistry, University of Delaware, Newark, Del. (U.S.A.)

PETRAS V. AVIZONIS*
JOHN C. WEISTON, JR.

[1] S. P. DATTA AND H. HARRIS, *J. Physiol.*, 114 (1951) 39P.
[2] R. G. WESTALL, *Biochem. J.*, 55 (1953) 244.
[3] S. TRIPPETT, *J. Chem. Soc.*, (1957) 1929.
[4] H. H. TALLAN, S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 211 (1954) 927.
[5] P. B. HAMILTON, *Anal. Chem.*, 30 (1958) 914.

Received April 2nd, 1959

* This report is based upon a dissertation presented for the degree of Master of Science in the Department of Chemistry, University of Delaware.