Distribution and Excretion of Carnitine in the Rat

SVEN LINDSTEDT and GÖRAN LINDSTEDT

Department of Chemistry I, Karolinska Institutet, Stockholm 60, Sweden

Carnitine is widely distributed in Nature. The highest concentration — 35 mg/g dry weight — has been found in muscles of the horseshoe-crab (*Limulus polyphemus*) while mammalian muscle contains about 1 mg/g dry weight. The lack of a simple and specific quantitative method has hampered investigations on the distribution and excretion of carnitine. Available data have mostly been obtained with a bioassay using the meal worm (*Tenebrio molitor*) which has an absolute nutritional requirement for carnitine (See Ref.[1] for review). Strack has used a method in which carnitine is converted to the methyl ester of N-trimethylaminocrotonic acid which is assayed on frog rectus muscle. With this method he has determined the distribution of carnitine in rats up to 3 h after administration of 2—10 mg (lethal dose) per g body weight [2].

We have prepared (—)-carnitine-[methyl-^{14}C] and DL-carnitine-[carboxy-^{14}C] of high specific activity which has been used for a study on the distribution and excretion of carnitine administered in doses within the physiological range.

Experimental. DL-*Carnitine-[carboxy-^{14}C]*, 3.3 μC/mg, was prepared by methylation of DL-β-hydroxy-γ-aminobutyric acid-1-^{14}C.

(—)-*Carnitine-methyl-^{14}C*, 71.5 μC/mg, was made by the methylation of (—)-β-hydroxy-γ-aminobutyric acid prepared by resolution of the optically inactive acid *via* the strychnine and brucine salts. Details of these procedures will be published separately [3].

The labelled compounds were dissolved in 0.5 ml of water and given intraperitoneally to rats of the Sprague-Dawley strain. Total radioactivity in the expired carbon dioxide was determined by drawing the expiratory air through an ionization chamber (Frieseke & Hoepfner GmbH, FH 56/23) with continuous registration of the activity level. Urine and feces were collected daily and the activity determined by plating aliquots on aluminium planchets and counting in a windowless proportional counter (Frieseke & Hoepfner GmbH, FH 407 A). The rats were killed by a blow on the head, the tissues homogenized and extracted with 5 % trichloroacetic acid (TCA). Radioactivity was determined on aliquots after extraction of the TCA with ether.

Results and discussion. Data on the distribution of isotope after administration of labelled carnitine are given in Table 1. Paperchromatography of a TCA-extract (ethanol/ammonia/water, 90/5/5) from muscle after administration of (—)-carnitine-[methyl-^{14}C] showed only one labelled compound with the same mobility as carnitine. Carnitine was initially distributed over the whole animal with about the same

Table 1. Distribution of isotope after administration of (—)carnitine-[methyl-^{14}C] and DL-carnitine-[carboxy-^{14}C]

Tissue	% of administered dose per g tissue wet weight				
	(—) carnitine-[methyl-^{14}C]			DL-carnitine-[carboxy-^{14}C]	
	Rat 1. 223 g, 7 h after administration of 1.2 mg	Rat 2. 183 g, 7 d after administration of 1.1 mg	Rat 3. 156 g, 7 d after administration of 1.0 mg	Rat 4. 125 g, 36 h after administration of 2.2 mg	Rat 5. 225 g, 10 d after administration of 2.2 mg
Brain	0.05	0.03	0.04	—	0.03
Liver	0.64	0.06	0.07	0.54	0.09
Kidney	0.68	0.20	0.21	1.00	0.09
Spleen	0.55	0.15	0.14	0.44	0.12
Lung	—	0.18	0.13	—	—
Heart	0.58	0.67	0.45	1.45	0.28
Skeletal muscle	0.18	0.29	0.24	0.70	0.17

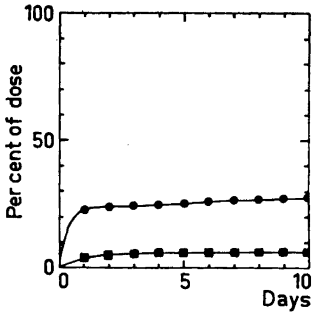

Fig. 1. Urinary excretion of isotope after administration of (−)-carnitine-[methyl-^{14}C] (1.1 mg) ■—■ and DL-carnitine-[carboxy-^{14}C] (2.2 mg) ●—●.

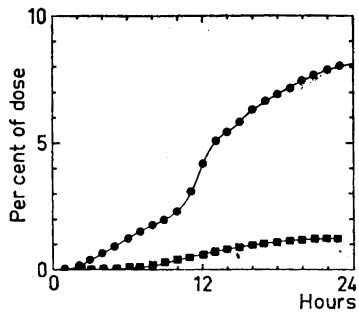

Fig. 2. $^{14}CO_2$ in expiratory air after administration of (−)-carnitine [methyl-^{14}C] (1.7 mg) ■—■ and DL-carnitine-[carboxy-^{14}C] (4.4 mg) ●—●.

concentration in all tissues except brain. After 7 and 10 days the highest concentration was found in heart muscle followed by skeletal muscle. Fritz [4] has shown that carnitine has a stimulatory effect on the oxidation of long chain fatty acids in muscle homogenates which is most pronounced with heart muscle preparation. The high uptake of administered carnitine by heart muscle may support his hypothesis that carnitine plays a role in the uptake of unesterified fatty acids by perifereal tissues, since heart is known to fill its energy requirements largely by oxidation of fatty acids.

The results obtained with (−)-carnitine showed that the amount present in the body after 7 days was approximately the same as after 7 h which indicates a slow biological turn-over of (−)-carnitine.

Figs. 1 and 2 illustrate the excretion of isotope in urine and expiratory CO_2 after administration of labelled carnitine. When (−)-carnitine-[methyl ^{14}C] was used 2−3 % was recovered as CO_2 in a 24 h period and less than 10 % in the urine in 10 days. After the first day the urinary excretion of activity remained at a fairly constant daily level of 0.1−0.2 % of the administered dose. When DL-carnitine-[carboxy-^{14}C] was given more of the isotope was recovered in the urine (30−40 %) and as $^{14}CO_2$ (10−15 %).

The experiments have demonstrated that when a small dose of natural carnitine is administered it is retained in the body over a long period. The slow biological turnover of carnitine may expain difficulties encountered in studies on the biosynthetic and degradative pathways of carnitine *.

This work has been supported by a grant from *Konung Gustaf V:s 80-årsfond.*

1. Fraenkel, G. and Friedman, S. *Vitamins and Hormones* **15** (1957) 74.
2. Strack, E., Rotzsch, W. and Lorenz, I. *Protides of the Biological Fluids, Proc. 7th Colloq. Bruges 1959*, p. 235.
3. Lindstedt, S. and Lindstedt, G. *Arkiv Kemi. To be published.*
4. Fritz, I. B. and Kaplan, E. *Protides of the Biological Fluids, Proc. 7th Colloq., Bruges 1959*, s. 252.

Received March 21, 1961.

* *Note added in proof.* Since completion of this work a paper has appeared in which the half-life of carnitine is estimated to 67 days in the rat. (Wolf, G. and Berger, C. R. A. *Arch. Biochem. Biophys.* **90** (1961) 360).