ON THE BIOSYNTHESIS AND DEGRADATION OF CARNITINE.[x)]

G. Lindstedt and S. Lindstedt.

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

Received October 2, 1961

Recent work (Wolf and Berger 1961; Lindstedt and Lindstedt 1961) has shown that the turnover of carnitine in the rat is very slow which could explain previous difficulties (Fraenkel and Friedman 1957) in demonstrating carnitine synthesis in vivo.

Wolf (1961) and Bremer (1961) have presented evidence that administration of labeled methionine results in the formation of labeled carnitine in the rat. The origin of the carbon chain of carnitine has been the subject of much speculation (Fraenkel and Friedman 1957; Cantoni 1960). Recent reports on the biological hydroxylation of γ-aminobutyric acid to β-hydroxy-γ-aminobutyric acid and on the occurence of this acid in brain (Hayashi 1959; Seo 1957) lent support to a hypothesis involving this acid as an intermediate. Attempts to verify these reports have however been unsuccessful (Lindstedt, Mitoma and Pisano 1960). When labeled β-hydroxy-γ-aminobutyric acid was prepared and administered in large doses to rats no formation of carnitine could be demonstrated.

Linneweh (1929) fed γ-butyrobetaine to dogs and demonstrated an increased excretion of carnitine in urine. He proposed a β-oxidation mechanism and suggested the further degradation of carnitine to glycine betaine.

We now want to report isotope experiments which demonstrate these pathways in carnitine metabolism.

x) Supported by a grant from Konung Gustaf V:s 80-årsfond.

Biosynthesis. When γ-butyrobetaine-1-C^{14} was injected intraperitoneally into a rat 40 % of the activity was excreted in the urine in 30 hours. Ion exchange chromatography (Fig. 1) showed that 50 % appeared as carnitine, while the rest was unchanged γ-butyrobetaine. The carnitine was further identified by several procedures (see below). 50 % of the administered activity was recovered by extraction of different tissues with TCA. Analysis of these extracts (liver, heart, muscle, carcass) by ion exchange chromatography showed that 98 % of the activity appeared as carnitine and therefore a total of at least 70 % of the injected γ-butyrobetaine had been converted to carnitine in this experiment. No glycine betaine was found. In preliminary experiments with whole homogenates of liver and kidney we have obtained conversion of γ-butyrobetaine to carnitine in liver homogenates.

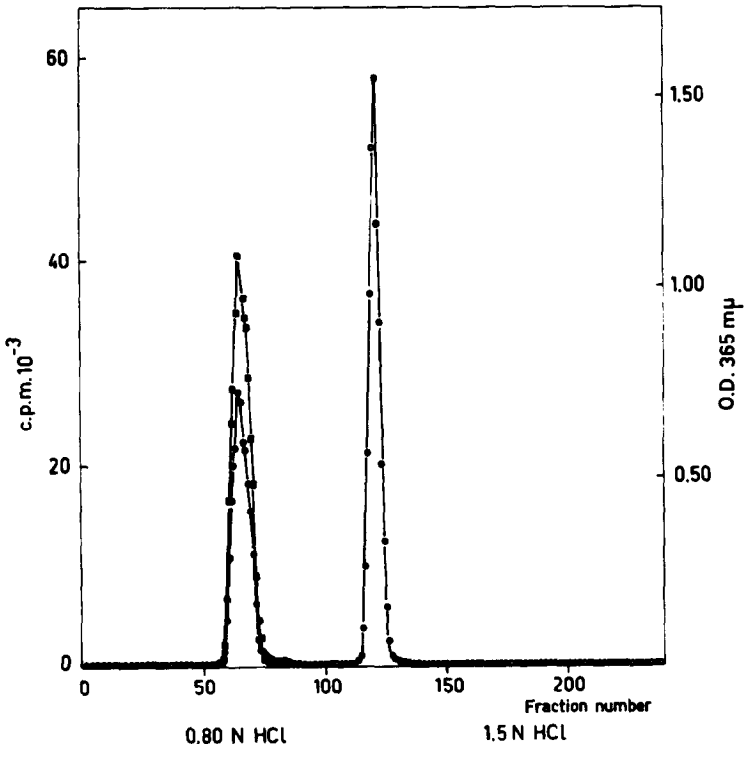

Fig. 1. Separation on Dowex-50 column of carnitine and γ-butyrobetaine in urine after administration of γ-butyrobetaine-1-C^{14} to a rat. ○ radioactivity; ■ periodide reaction. Carnitine appears in fractions 60-72, γ-butyrobetaine in fractions 116-126. 20 mg carrier carnitine added.

Since γ-aminobutyric acid seemed the most likely precursor of γ-butyrobetaine this amino acid was administered intraperitoneally into rats. In one experiment where 50 μC were used no formation of carnitine could be demonstrated though a conversion of 0.01 % of the dose should have been detected.

Degradation. Urine samples collected from several rats fed (-)carnitine-[methyl-C^{14}] of high specific activity were pooled and fractionated on a Dowex-50 column. Carnitine accounted for 97 % of the activity while the remaining 3 % appeared as trimethylamine oxide (TMAO). No other metabolites were found.

Aurich and Lorenz (1959) have shown that a strain of P. pyocyaneus (NCTC A 7244) can utilize carnitine as the sole energy source. Under our conditions we were unable to obtain significant growth on a synthetic medium supplemented with carnitine. Addition of choline however, resulted in satisfactory growth. When labeled (-)carnitine-[methyl-C^{14}] was added to the medium one major metabolite was identified as glycine betaine by paper chromatography (Fig. 2) and on ion exchange columns. The material that appears at the origin in Fig. 2 has not been identified.

Experimental procedures. (-)carnitine-[methyl-C^{14}] and DL-β-hydroxy-γ-aminobutyric acid-1-C^{14} were the preparations used in previous work (Lindstedt and Lindstedt 1961; Lindstedt and Lindstedt in press). γ-butyrobetaine-1-C^{14} was prepared by the methylation of γ-aminobutyric acid-1-C^{14} (1.85 mC/mmole). The compounds were administered intraperitoneally into rats weighing 60-100 g. Tissues were homogenized, extracted with TCA and separated on columns of Dowex-50 (Friedman, Mc Farlane, Bhattacharyya and Fraenkel 1955). Fractions were analyzed for quarternary ammonium compounds with the periodide method either used as a spot test or in a quantitative modification (Ward, Christianson, Dimler and Senti 1960) and for amino acids with the ninhydrin method. Radioactivity was determined by plating aliquots on glass planchets. For identification

of metabolites paper chromatograms were run with authentic material and scanned for radioactivity. Four solvent systems were used: ethanol/ammonia/water 90:5:5; butanol/ethanol/water 4:1:5; phenol/butanol (water saturated) 20:7, phenol saturated with 0.1 N HCl. Spots were visualized by dipping in 1% solution of iodine in ether (Brante 1949).

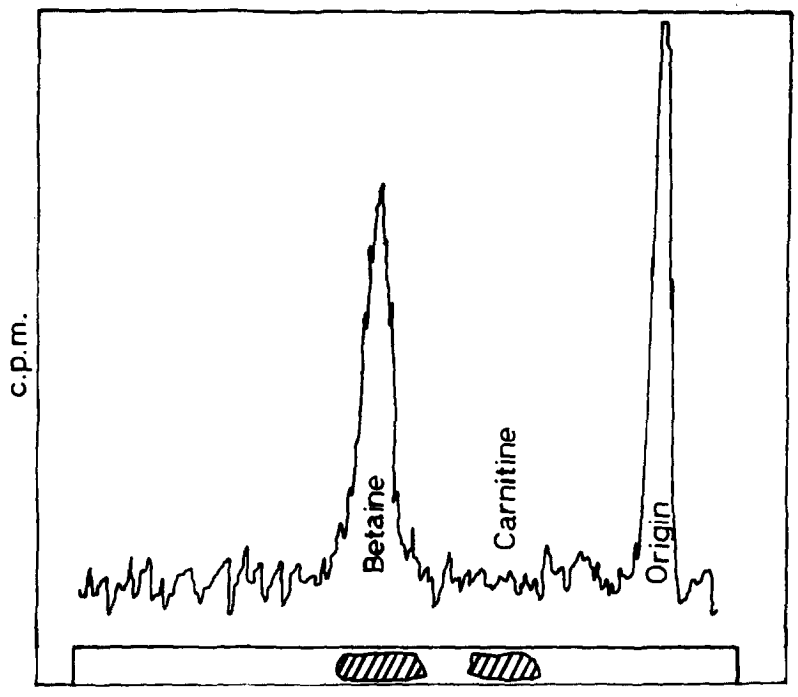

Fig. 2. Distribution of C^{14} on paper chromatogram (ethanol/ammonia/water 90/5/5) of growth medium after a 3 days incubation of P. pyocyaneus with (-)carnitine-[methyl-C^{14}]. Carrier glycine betaine and carnitine added. Spots developed with iodine.

For qualitative analysis of fractions from ion exchange columns thin layer chromatography was generally superior to paper chromatography due to higher resolving power and higher sensitivity (1-2 /ugs of bases required) (Eneroth, Lindstedt and Lindstedt). For location of radioactive material the layer was scraped off and eluted with 80% methanol.

Final identification of the metabolites was based on recrystallization to constant specific activity with carrier material.

The present series of experiments have confirmed the reaction sequences proposed by Linneweh[x]. The only metabolite of carnitine that could be found in rat urine was TMAO, which most likely originates from trimethylamine split off by bacterial action in the gut. The possibility that small amounts of glycine betaine are formed in animal as well as in bacterial metabolism of carnitine cannot be excluded, since the latter compound is rapidly metabolized in itself.

Though γ-butyrobetaine is suggested as a natural precursor of carnitine the mechanism for its formation in the animal body is still obscure. Experiments with N-monemethyl-γ-aminobutyric acid should be helpful in deciding if a successive methylation of this acid could take place. Work on this problem and on the enzyme mechanisms in carnitine metabolism will be published subsequently.

REFERENCES.

Aurich, H. and Lorenz, I.: Acta Biol. Med. Germ. 3, 272 (1959).
Brante, G.: Nature 163, 651 (1949).
Bremer, J.: Biochim. Biophys. Acta 48, 622 (1961).
Bremer, J.: Biochim. Biophys. Acta, in press.
Cantoni, G.L.: in Comparative Biochemistry, Ed. Florkin, M., Matson, H.S. vol. 1, 181. Acad. Press 1960.
Eneroth, P., Lindstedt, G. and Lindstedt, S.: to be published.
Fraenkel, G. and Friedman, S.: in Vitamines and Hormones 15, 74 (1957).
Friedman, S., McFarlane, J.E., Bhattacharyya, P.K. and Fraenkel, G.: Arch. Biochem. Biophys. 59, 484 (1955).
Hayashi, T.: J. Physiol. 145, 579 (1959).
Lindstedt, S., Mitoma, C. and Pisano, J.: reported by Mitoma, C. in Inhibition in the Nervous System and γ-aminobutyric acid. Pergamon Press, p. 236 (1960).
Lindstedt, S. and Lindstedt, G.: Acta Chem. Scand. 15, 701 (1961).
Lindstedt, S. and Lindstedt, G.: Arkiv Kemi, in press.
Linneweh, W.: Hoppe & Seyler Zeitschr. Physiol. Chem. 181, 42 (1929).
Seo, S.: Med. J. Osaka Univ. 7, 833 (1957).
Ward, J.S., Christianson, P.D., Dimler, R.J. and Senti, F.R.: Anal. Chem. 32, 870 (1960).
Wolf, G. and Berger, C.R.A.: Arch. Biochem. Biophys. 92, 360 (1961).

x) While this work was in progress we learnt that Dr. J. Bremer, Oslo, has also obtained evidence for the formation of carnitine from γ-butyrobetaine (Bremer 1961 b).