sis which has the same specificity towards B_{12}.

Two recent reports suggest that the methyl-malonyl-CoA—succinyl-CoA conversion in *O. malhamensis* is in the direction of succinyl-CoA formation(8,9). Thus if any compound in this pathway is active in replacing B_{12}, one would expect succinate rather than methyl-malonate to be active, but our unpublished experiments exclude this possibility. The importance of the B_{12}-dependent methyl-malonyl CoA → succinyl CoA reaction in *O. malhamensis* has been questioned. In successive notes, the same authors suggested first that succinate formation is not the reaction by which Vit. B_{12} controls growth(8) and then that the major role of B_{12} may be in the propionate → succinate pathway(9). Our results favor the first interpretation.

Our growth experiments indicate that the reactions responsible for the B_{12} growth requirement in *E. gracilis* and *O. malhamensis* are still to be identified. One possible area for investigation is the series of reactions resulting in the formation of lecithin. Lecithin was strikingly effective in reversing benzimidazole-caused growth inhibition in *O. malhamensis* in a medium which contained B_{12} plus all the compounds now recognized as products of B_{12}-catalyzed reactions(6).

Summary. The following compounds do not either replace or spare the growth requirement for Vit. B_{12} of either *E. gracilis* or *O. malhamensis*: DL-β-methyl-aspartate, L-threo-β-methyl-aspartate, ureido-β-methyl-aspartate, N-acetyl-L-threo-β-methyl-aspartate, and methyl-malonate. Inhibition of growth of *E. gracilis* by benzimidazole is not annulled by β-methyl-aspartate.

1. Isenberg, H. D., Seifter, E., Berkman, J. I., *Biochim. Biophys. Acta,* 1960, v39, 187.

2. Ford, J. E., Hutner, S. H., in *Vitamins and Hormones,* Academic Press, New York, 1955, p102.

3. Barker, H. A., Weissbach, H., Smyth, R. D., *Proc. Nat. Acad. Sci.* (U.S.A.), 1958, v44, 1093.

4. Smith, R. M., Monty, K. J., *Biochem. Biophys. Res. Comm.,* 1959, v1, 105.

5. Nathan, H. A., in *Microbiological Assays,* Academic Press, New York, 1962, in press.

6. Nathan, H. A. Funk, H. B., *Am. J. Clin. Nutrit.,* 1959, v7, 375.

7. Isenberg, H. D., personal communication.

8. Arnstein, H. R. V., White, A. M., *Biochem. J.,* 1961, v79, 3P.

9. White, A. M., Arnstein, H. R. V., *ibid.,* 1961, v79, 10P.

Received October 30, 1961. P.S.E.B.M., 1962, v109.

Studies on Lipid Metabolism in Mice Treated with β-Hydroxy, γ-Betaine-Butyric Acid. (27157)

WILLIAM L. MILLER AND JOHN J. KRAKE (Introduced by Jacob C. Stucki)
Department of Metabolic Diseases, The Upjohn Co., Kalamazoo, Mich.

Little is known concerning the physiological or biochemical function of β-hydroxy, γ-betaine-butyric acid (carnitine)(1). Recent reports have shown that carnitine can stimulate the oxidation of palmitic acid by tissue preparations *in vitro*(2-5). It has been suggested that its site of action is in early stages of fatty acid metabolism where it enhances transfer of long chain fatty acids to an enzyme complex(6). The purpose of this study was to evaluate the action of carnitine on fatty acid oxidation by intact mice and fatty acid pattern or quantities of depot fat in non-obese and obese mice respectively.

Materials and methods. Male albino mice weighing 18-23 g, obtained from the Upjohn colony (Rockland Farm Swiss mouse ancestry) were used. For respiratory studies mice were fasted 15 hours (overnight) before use. DL-carnitine-hydrochloride* (DLC) was dissolved in deionized water and either injected intraperitoneally or given orally by stomach tube, ½ or 1 hour, respectively, before C^{14} compounds were injected. The C^{14}-

* International Minerals and Chemical Corporation, Skokie, Illinois.

carboxyl labeled long chain fatty acids and tripalmitin were incorporated into Lipomul† and injected into a tail vein. Each mouse received 0.2 ml of the emulsion which contained 7.8×10^4 cpm C^{14}-1-tripalmitin (carboxyl labeled), 1.7×10^5 cpm C^{14}-1-palmitic acid or 1.7×10^5 cpm C^{14}-1-oleic acid. Other mice were injected intraperitoneally with 0.5 ml aqueous solutions of 4.4×10^5 cpm C^{14}-1-sodium butyrate, 3.0×10^5 cpm C^{14}-1,3-glycerol, 5.5×10^5 cpm C^{14}-2-sodium acetate, 1.7×10^5 cpm C^{14}-glucose (uniformly labeled), 1.7×10^5 cpm C^{14}-2-glycine, or 1.5×10^5 cpm C^{14}-1-DL-leucine. Each group (control or DLC treated) of 6 mice was placed in a glass metabolic chamber from which the respired $C^{14}O_2$ was collected and then counted as $BaC^{14}O_3$(7).

In another series, non-obese mice were injected intraperitoneally with 100 mg/kg DLC daily for 4 weeks (food and water *ad libitum*). Mice were weighed, sacrificed, and 250 mg (wet weight) of epididymal fat taken from each mouse for fatty acid analysis. Samples were homogenized separately with 2.5 ml of deionized water and extracted twice with 5 ml of Skelly Solve "B" to recover lipids. Extracts were dried over Na_2SO_4 and concentrated to dryness using a stream of nitrogen and partial vacuum. Residues were saponified and transesterified to form methyl esters of the fatty acids(8), which were used with conventional gas chromatographic technics for fatty acid determinations. Obese mice were prepared by intraperitoneal injection of 1 mg/g body weight of gold-thioglucose(9) as Solganal‡, 3 months prior to initiation of DLC treatment. At beginning of the experiment, one group of obese mice received 250 mg/kg of DLC orally by stomach tube daily for 4 weeks. At this time, however, the daily dose was increased to 500 mg/kg given in divided doses twice a day for an additional 4 weeks. These mice were given a fluid diet (12.5 calories/5 ml) daily (Table I) in cups so that food consumption could be easily determined. This amount of food was previously found to be slightly less than the mice would eat. Mice were sacrificed by decapitation, liver and intestinal tracts removed, and the carcasses analyzed for lipid, protein, and water content (10).

TABLE I. Low Carbohydrate Fluid Diet.

Constituent	Quantity (g)	Constituent	Quantity (g)
Cellu flour	25	Casein	160
Salt mix, USP IV, #2	25	Starch	160
Dried liver powder	25	Dextrin	160
Skim milk "	200	Soya bean oil	60
Brewer's yeast	100	Methionine	3
Sucrose	170	Water	1000

To the above mixture 10 mg vit. K (2-methyl-1, 4-naphthoquinone), 200 mg α-tocopherol, 1 g ascorbic acid, and 3 ml Zymadrops (Upjohn trademark for a complete vitamin mixture) were also added. Percent of total calories for protein, carbohydrate, and fat was 21, 49, and 30, respectively. Each ml contained 2.5 calories.

Results and discussion. Respiration Studies. Cumulative C^{14} specific activities of CO_2 collected during oxidation of labeled compounds are either shown (Table II) or mentioned (Table II, footnote). No changes were observed in the total respiratory CO_2 due to DLC administration in all experiments. Fasted mice injected with DLC oxidized both C^{14}-tripalmitin and C^{14}-palmitic acid at an increased rate; peak $C^{14}O_2$ specific activity increases were 25% by the first hour and 50% by the second hour, respectively. Carnitine did not appear to affect the oxidation of C^{14}-oleic acid, glycerol, sodium butyrate or acetate, glucose, glycine or DL-leucine. Recently, it was reported(11) that DLC did not stimulate the oxidation of short chain fatty acids *in vitro*, which agrees with the negative results we obtained using sodium butyrate and acetate. On the other hand, a stimulation of oleic acid oxidation was observed *in vitro* while we did not obtain this result in our *in vivo* studies. The reason for this difference is not clear. Carnitine given orally produced a marked and sustained effect on C^{14}-tripalmitin oxidation; specific activity of $C^{14}O_2$ increased as much as 40 to 90% during the 5-hour observation. Increasing the oral dose from 250

† Upjohn trademark for intravenous cottonseed oil emulsion.

‡ Schering Corp. trademark for suspension of gold-thioglucose in sesame oil.

TABLE II. Effect of Injected Carnitine on Oxidation of C^{14}-Labeled Fatty Acids, Sodium Butyrate, and Glycerol by Fasted Mice.*

Group	Cumulative C^{14} specific activity at† ½ hr	1 hr	2 hr	3 hr	5 hr
C^{14}-1-tripalmitin					
Control	9.1 ± 1.1§	9.6 ± .9	10.1 ± 1.4	9.9 ± 1.4	9.7 ± 1.8
Carnitine‡	10.2 ± 1.9	12.1 ± 2.0	12.6 ± 1.4	11.0 ± 1.1	10.0 ± .8
C^{14}-1-palmitic acid					
Control	110 ± 26.1	84 ± 19.5	62 ± 18.2	51 ± 16.3	38 ± 7.5
Carnitine	158 ± 13.8	118 ± 18.8	96 ± 9.4	80 ± 9.4	57 ± 1.3
C^{14}-1-oleic acid					
Control	78 ± 10.5	57 ± 6.0	44 ± 2.4	34 ± 1.1	27 ± 1.1
Carnitine	78 ± 15.7	63 ± 7.9	47 ± 6.9	37 ± 3.8	28 ± 2.4
C^{14}-1-sodium butyrate					
Control	353 ± 53.1	255 ± 37.2	174 ± 35.3	130 ± 25.1	96 ± 17.4
Carnitine	370 ± 69.2	244 ± 35.1	156 ± 36.7	118 ± 34.1	90 ± 19.2
C^{14}-1,3-glycerol					
Control	45 ± 7.0	48 ± 4.8	38 ± 6.1	30 ± 4.5	25 ± 2.7
Carnitine	55 ± 11.5	51 ± 6.0	40 ± 3.0	32 ± 2.0	25 ± 2.6

* Values obtained from 18 mice/group (control or carnitine-treated) in 3 separate runs of 6 mice/chamber. Carnitine did not alter the C^{14} specific activity of CO_2 from the oxidation of C^{14}-2-sodium acetate, C^{14}-UL-glucose, C^{14}-2-glycine, or C^{14}-1-DL-leucine (see *Materials and Methods*). Amount of total exhaled CO_2 was unchanged by carnitine in all experiments.

† Counts/min./mg $BaCO_3$.

‡ 100 mg/kg carnitine intraper.

§ Stand. error of mean.

mg/kg to 1 g/kg produced no significantly greater stimulation (Table III).

Fatty acid and carcass analysis. Non-obese mice treated with DLC for 4 weeks did not lose weight, nor did they appear to lose depot fat (Table IV, footnote). No marked differences were found in comparing per cent composition of major fatty acids of epididymal pads of control mice with that of treated mice (Table IV). Carnitine did not markedly stimulate the utilization of carcass fat in the obese mice (Table V). Treated mice contained only slightly less lipid and slightly more protein on a percentage basis than did control mice. Treated mice weighed less, however, at the end of experiment than did control mice (Table V, footnote). The weight decrease was probably due to loss of lipid and loss of body water from 69.2 down to 65.8% as well as decreased food consumption (Table V, footnote).

Summary. Administration of DL-carnitine hydrochloride (DLC) markedly increased the oxidation of C^{14}-palmitic acid injected as such or as the triglyceride, but did not alter total CO_2 output of fasting mice. Oxidation of C^{14}-labeled oleic acid, glycerol, sodium butyrate or acetate, glucose, glycine or DL-leucine was not increased. Long term administration of DLC neither altered the per cent composition of the major fatty acids in epididymal lipids of non-obese mice, nor was it able appreciably to decrease the carcass lipids of gold-thioglucose obese mice.

TABLE III. Effect of Oral Carnitine on Oxidation of C^{14}-1-Tripalmitin by Fasted Mice.*

Group	Dose, mg/kg	Cumulative C^{14} specific activity at† ½ hr	1 hr	2 hr	3 hr	5 hr
Controls	—	8.6 ± 2.6	9.0 ± 1.4	8.6 ± 2.8	8.4 ± 1.5	8.1 ± 2.1
Carnitine	250	12.8 ± 1.6	14.0 ± 2.6	15.0 ± 1.6	16.0 ± 1.8	14.8 ± 1.4
"	1000	11.6 ± .5	16.8 ± 1.6	16.8 ± 2.8	16.6 ± 2.6	15.2 ± 2.8

* Values obtained from 18 mice/group as per Table I. Carnitine did not alter total amount of CO_2 exhaled.

† Counts/min./mg $BaCO_3$.

TABLE IV. Effect of Carnitine on Fatty Acid Composition of Mouse Epididymal Fat.*

Group	Fatty acid (mole %)†					
	Palmitic	Oleic	Linolenic	Stearic	Palmitoleic	Myristic
Controls (10)	27.1 ± .6	38.9 ± .6	23.3 ± .1	1.6 ± .1	7.2 ± .6	1.8 ± .2
Carnitine (10)	26.5 ± .6	39.0 ± .5	23.0 ± .2	2.5 ± .2	7.3 ± .6	1.8 ± .2

* 100 mg/kg carnitine daily for 4 wk. Final total body and epididymal pad (wet) weights for controls were: 30 ± 1 g and 241 ± 20 mg; for carnitine-treated mice: 29 ± .8 g and 256 ± 18 mg, respectively.

† Only trace amounts of lauric and linolenic acids were found in all samples.

TABLE V. Carcass Composition of Gold-Thioglucose Obese Mice Given Carnitine.*

Group†	Carcass analysis at 8 wk		
	Lipids (% dry wt)	Protein (% dry wt)	Water, %
Controls (7)	70 ± .9	13.7 ± .2	69.2 ± 1.1
Carnitine (6)	67 ± 1.3	16.1 ± .3	65.8 ± 1.2

* 250 mg/kg/day oral carnitine for 4 wk, 500 mg/kg in divided doses for 4 additional wk.

† In 8 wk, control obese mice each increased in mean wt from 46 ± .9 to 63 ± .8 g; carnitine treated mice increased from 46 ± 1.1 to 58 ± 1.6 g. Food consumption decreased about 15% during the last 10 days of experiment for mice given carnitine.

The authors are indebted to Dr. C. E. Meyer for help in preparing fat emulsions; to Dr. G. R. Umbreit for gas chromatographic analysis of lipid samples; and to Dr. W. E. Dulin, Mr. G. H. Lund, and Mrs. R. L. Kupsis for carcass analyses.

1. Harris, R. S., Marriau, G. F., Drimann, D. V., *Vitamins and Hormones,* 15, New York, Academic Press, 1957, p73.

2. Fritz, I. B., McEwen, B., *Science,* 1959, v129, 334.

3. ———, *Acta Physiol. Scand.,* 1955, v34, 367.

4. ———, *Am. J. Physiol.,* 1957, v190, 449.

5. ———, *The Physiologist,* 1958, v1, 25.

6. ———, *Am. J. Physiol.,* 1959, v197, 297.

7. Miller, W. L., Jr., Krake, J. J., VanderBrook, M. J., *J. Pharm. Exp. Therap.,* 1957, v119, 513.

8. Stoffel, W., Chu, F., Akrens, E. H., Jr., *Anal. Chem.,* 1959, v31, 307.

9. Waxler, S. H., Brecher, G., *Am. J. Physiol.,* 1950, v162, 428.

10. Kochakian, C. B., Stettner, C. E., *ibid.,* 1948, v155, 255.

11. Fritz, I. B., Kaplan, E., *Fed. Proc.,* Part 1, 1960, v19, 146.

Received October 30, 1961. P.S.E.B.M., 1962, v109.

Effect of MER-29 on Egg Production in the Chicken.* (27158)

T. L. BURGESS, C. L. BURGESS AND J. D. WILSON† (Introduced by M. D. Siperstein)

Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas

Several studies in rats and man have conclusively shown that 1-[p-(β-diethylaminoethoxy) - phenyl] - 1 - (p - tolyl) - 2 - (p-chlorophenyl) ethanol (MER-29) causes a depression in serum and tissue cholesterol by inhibiting cholesterol synthesis(1,2), as a result of which 24-dehydrocholesterol (desmosterol) accumulates in serum and tissues(3-5). Despite the regular depression of cholesterol concentration, the effects on total sterol levels vary: the tissue sterols are usually normal, whereas blood sterol concentration may be low(4).

Since the chicken egg may contain as much as 0.25-0.3 g of cholesterol(6), the effect of MER-29 on egg production and egg sterol concentration has been studied in the chicken. The experiments reported here demonstrate that MER-29, when fed in large quantities, results in an almost complete replacement of egg cholesterol by desmosterol and that this

* Supported by grants from Am. Heart Assn. and Dallas Heart Assn.

MER-29 used in these experiments was a gift of Wm. S. Merrell Co.

† Established Investigator of Am. Heart Assn.