Turnover of Carnitine by Rat Tissues

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Radioactive carnitine, in the form of L-[methyl-³H]carnitine, was administered intravenously to male rats and the specific radioactivity of carnitine in blood plasma and 13 tissues was measured for 16 days. There was no evidence of metabolism of carnitine to other compounds. A compartmental analysis was made by comparing the variation with time of the specific radioactivity of each tissue with one of two models. Kidney, heart and epididymal fat were best represented as containing a single compartment of carnitine, whereas spleen, liver, lung, adrenal, prostate, seminal vesicle, pancreas, muscle, testis and brain were best represented in terms of two compartments each exchanging carnitine with blood plasma. Estimates were obtained of the turnover times of carnitine in the individual tissue compartments as well as the fluxes across each compartment boundary. Analysis of the variation in the specific radioactivity of carnitine in urine and blood plasma indicated an average total excretion rate of carnitine of 10.4 μ mol/day, of which about 3.2 μ mol was found in the urine.

The importance of carnitine in fatty acid metabolism is now well established and much effort is currently being devoted to a study of its mode of action at the subcellular level. In contrast, this report examines the inter-relationships between and within tissues of the whole animal with respect to their contents of carnitine and their exchange of carnitine with the blood plasma. Most tissues derive carnitine from the bloodstream, which in turn is supplied from the diet or from synthesis in the liver (Häigler & Broquist, 1974).

The rates of uptake of injected radioactive carnitine by different organs have been presented (Yue & Fritz, 1960, 1962; S. Lindstedt & G. Lindstedt, 1961; Mehlman & Wolf, 1962), as have estimates of the turnover of carnitine in the body (S. Lindstedt & G. Lindstedt, 1961; Wolf & Berger, 1961; Khairallah & Mehlman, 1965; Therriault & Mehlman, 1965*b*; Mehlman *et al.*, 1969, 1971). In most cases, animals were given a mixture of the naturally occurring L-carnitine and the unnatural D-carnitine, and the results were interpreted on the assumption that the D isomer is selectively excreted by the kidney soon after administration (S. Lindstedt & G. Lindstedt, 1961). Further, in many cases large doses of carnitine were administered owing to the low specific radio-

activity of the carnitine available and this added additional uncertainty to the interpretation of the results. The use in the present study of the natural isomer, L-carnitine, of high specific radioactivity has helped to overcome some of these uncertainties.

Materials and Methods

Chemicals

Chemicals were of analytical-reagent grade where available and were supplied by Fisher Scientific Co., Medford, Mass., U.S.A. Other chemicals were obtained as follows: L-carnitine hydrochloride from General Biochemicals, Chagrin Falls, Ohio, U.S.A.; CoA and carnitine acetyltransferase (EC 2.3.1.7) from Boehringer Mannheim Corp., New York, N.Y., U.S.A.; 5,5'-dithiobis-(2-nitrobenzoic acid) from Sigma Chemical Co., St. Louis, Mo., U.S.A.; Dowex 50(X8; 100–200 mesh; H⁺ form) from Bio-Rad Laboratories, Richmond, Calif., U.S.A.; DL-[methyl-¹⁴C]carnitine hydrochloride (22.5 mCi/mmol) from International and Nuclear Corp., Waltham, Mass., U.S.A.; [³H]toluene (0.1 μ Ci/mmol) from New England Nuclear Corp., Boston, Mass., U.S.A.

Treatment of animals

Details of the preparation, identification and administration of L-[methyl-³H]carnitine hydrochloride, and of the experimental animals, have been

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given by Brooks *et al.* (1973). Essentially, 14 male rats (395 ± 30 g) housed at 25°C were injected intravenously with $18.7 \mu\text{Ci}$ (15.6 nmol) of L-[methyl- ^3H]-carnitine hydrochloride in $10 \mu\text{l}$ of 0.15 M-NaCl . The animals were subsequently killed by decapitation at times up to 16 days after the administration of labelled carnitine. Blood was collected into heparinized tubes and the plasma separated by centrifugation. Tissues were dissected out and frozen in liquid N_2 within 5 min of the death of the animal.

Preparation of extracts

The frozen tissue was pulverized in a percussion mortar and a weighed amount (about 0.5 g) of the frozen powder was transferred to a tube containing 5 ml of 0.1 M-KOH with 1 mM-EDTA . The fragmented tissue was digested at 50°C for 4 h, cooled to 0°C , acidified with $50 \mu\text{l}$ of 60% (w/v) HClO_4 , and centrifuged. The supernatant was applied to a column ($0.6 \text{ cm} \times 6 \text{ cm}$) of Dowex 50 and the column was washed with $3 \times 5 \text{ ml}$ of water. Carnitine was then eluted from the column with 20 ml of 0.5 M-NH_3 . This eluate was freeze-dried and made up in 1 ml of water. When DL-[methyl- ^{14}C]carnitine was taken through the procedure no significant losses were apparent.

The total daily output of urine was collected every 24 h from animals housed in cages equipped with collection facilities suitable for obtaining the urine free from faeces. The urine was centrifuged to remove particulate matter and diluted to 35 ml. A 4 ml portion was purified in the same manner as for tissue extracts.

Analysis of extracts

Samples of the purified carnitine fraction were used for the determination of carnitine (Marquis & Fritz, 1964), and of radioactivity by liquid-scintillation counting. To each sample 5 ml of scintillation fluid [toluene containing 4 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene per litre, mixed 2:1 (v/v) with Triton X-100] was added and counts were corrected to d.p.m. by internal standardization with [^3H]toluene.

That carnitine was the sole radioactive component present in the purified extracts from all tissues was confirmed in each of two chromatographic systems. First, the elution position of radioactivity from a column ($0.6 \text{ cm} \times 10 \text{ cm}$) of Dowex 50 developed at 25 ml/h with a linear gradient (150 ml of water plus 150 ml of 1.5 M-HCl) was identical with authentic carnitine; no other radioactive fractions were obtained. Secondly, the fractions containing radioactivity were pooled, freeze-dried, made up in 1 ml of water and portions were chromatographed on Whatman no. 1 paper developed with propan-1-ol- 18 M-NH_3 -water (17:1:3, by vol.). Carnitine was detected with I_2 vapour and the chromatogram then

cut into 1 cm strips which were counted to determine the distribution of radioactivity.

Analysis of data

Compartmental analysis was applied to the measurements of specific radioactivity of carnitine in the tissues to determine which of two simple mathematical models would satisfactorily simulate the observed behaviour of the tracer. Specific radioactivities were calculated by dividing d.p.m./unit mass of fresh tissue by nmol of total carnitine/unit mass of fresh tissue. Blood plasma was assumed to be the reservoir of labelled carnitine, and also to be the only path by which carnitine is transported from one tissue to another. One model was a two-compartment open system in which blood plasma exchanged carnitine with a single compartment in a second tissue. The other model was a three-compartment open system in which there was exchange between blood plasma and two independent compartments in the second tissue. Turnover time is the time taken for an amount of carnitine equal to the total carnitine in a compartment to pass in one direction across its boundary. The flux of carnitine equals the amount of carnitine transported into or out of a compartment in unit time. Full details of the models and the assumptions made in their use, as well as the numerical method of fitting them to the experimental data by using a computer, have been given by McIntosh & Lutwak-Mann (1972).

Estimates were made of the total rates of excretion of carnitine from the animals by application of the Stewart-Hamilton equation to the time-courses of specific radioactivity of carnitine in the urine and in the blood plasma (Shipley & Clark, 1972). The values of specific radioactivity of carnitine in the 24 h urine collections were summed to give the area under the curve of specific radioactivity against time until the last collection. An estimate was made of the area from this final point until infinite time by fitting a single exponential decay to the last part of the curve. The dose of labelled carnitine injected divided by the total area under the specific-radioactivity-time curve gave the total rate of output of carnitine from the animal by all routes. Similarly, the area under the curve of mean specific radioactivity in the blood plasma (Fig. 1a) was obtained as follows. The area under the curve between the time of the first sample (2 h) and infinite time was obtained from the measured exponential coefficients of the curve (see Table 2). By interpolating a single exponential decay between the calculated specific radioactivity in the blood plasma at the time of injection (derived from the dose injected, the body weight and an assumed blood plasma volume of $34 \text{ ml/kg body wt.}$) and the measured specific radioactivity at 2 h after injection, the area between zero time and 2 h was estimated. The dose

divided by the total area gave another measure of the excretion rate of carnitine.

Results

Carnitine content of tissues

The procedure used for the determination of carnitine was designed to extract the total tissue carnitine by hydrolysing any *O*-acyl carnitine to release the free compound (Pearson & Tubbs, 1964). The amounts of total carnitine measured in the tissues examined are presented in Table 1. Heart muscle was found to have the greatest content of carnitine, and epididymal fat the least, except for erythrocytes, which did not contain any detectable carnitine. The low amount of carnitine in blood plasma meant that the contamination of other tissues by blood did not significantly affect their measured contents of carnitine.

Compartmental analysis

The direct intravenous injection of radioactive carnitine resulted in the rapid distribution of radioactivity throughout the body. This rapid distribution was essential for subsequent compartmental analysis to be carried out. In order to conduct the analysis it was first necessary to describe mathematically the behaviour of the specific radioactivity of carnitine in the blood plasma, which represents the reservoir of the model system. It was found that the simplest satisfactory description of the behaviour of the specific radioactivity in this tissue was given by a

function consisting of the sum of four exponential terms. The values of the derived coefficients are given in Table 2. The necessity for more than two exponential terms stems from the fact that the specific radioactivity was followed over a long period (2h to 16 days). At any particular time, however, the shape of the curve is only significantly affected by two of the terms. The behaviour of the specific radioactivity of carnitine in blood plasma from 2 to 96h is shown in Fig. 1(a). It must be emphasized that the equation describing the specific radioactivity in the bloodstream is only a mathematical description of the curve and the values of the coefficients in the equation have no direct significance in terms of describing exchanges with the other tissues.

The variation of the specific radioactivity of carnitine in each tissue was then related to the behaviour of the specific radioactivity in the blood plasma. By comparing the goodness of fit of the experimental points to the theoretical curves produced by the computer, a choice was made between the two models (that is, between the two-compartment open system and the three-compartment parallel open system). This does not mean that the data may not in fact be better represented by other models. However, the precision of the data did not allow the testing of more complex models.

The variation with time of the specific radioactivity in each tissue is shown in Fig. 1. For reasons of clarity, the time scale is shown only up to 96h after injection. The curves represent the models as fitted by the computer. The derived compartment sizes, turnover times and flux rates are presented in Table 3. For kidney, heart muscle and epididymal fat the data were best represented by the two-compartment model, whereas the other tissues studied were more accurately described by the other model.

The small, slowly exchanging compartment in the liver and adrenal contributed little to the total tissue

Table 1. *Total carnitine in rat tissues*

Tissues were heated in 0.1M-KOH containing 1mM-EDTA to hydrolyse any *O*-acylcarnitine. Carnitine was determined by spectrophotometric assay with carnitine acetyltransferase. The results represent the mean $\pm$ S.D. from 14 determinations.

	Concn. of carnitine (μ mol/g fresh wt.)
Heart (ventricle muscle)	1.42 $\pm$ 0.16
Skeletal muscle (quadriceps)	1.03 $\pm$ 0.14
Kidney	0.930 $\pm$ 0.154
Lung	0.553 $\pm$ 0.106
Spleen	0.375 $\pm$ 0.066
Adrenal	0.269 $\pm$ 0.046
Liver	0.265 $\pm$ 0.113
Seminal vesicle	0.244 $\pm$ 0.022
Testis	0.191 $\pm$ 0.036
Prostate	0.186 $\pm$ 0.087
Pancreas	0.179 $\pm$ 0.029
Brain	0.117 $\pm$ 0.028
Blood plasma	0.087 $\pm$ 0.013
Epididymal fat	0.023 $\pm$ 0.008

Table 2. *Coefficients of the equation describing the specific radioactivity of carnitine in the blood plasma of rats after the administration of L-[methyl- 3 H]carnitine*

The equation has the form $SA_t = A_1 \exp(-B_1 t) + A_2 \exp(-B_2 t) + A_3 \exp(-B_3 t) + A_4 \exp(-B_4 t)$ where SA_t represents the specific radioactivity of carnitine in the blood plasma at time t after the injection of labelled carnitine. Equal weighting was given to the experimental points. The values of the coefficients are given below $\pm$ S.E.M. The experimental points and the fitted curve are shown in Fig. 1(a).

	(d.p.m./nmol)		(h $^{-1}$)
A_1	1880 $\pm$ 950	B_1	1.45 $\pm$ 0.29
A_2	1140 $\pm$ 68	B_2	0.265 $\pm$ 0.017
A_3	113 $\pm$ 23	B_3	0.0211 $\pm$ 0.0113
A_4	118 $\pm$ 23	B_4	0.00172 $\pm$ 0.00064

Fig. 1. Specific radioactivity of total carnitine in rat tissues after a single intravenous dose of L-[methyl- 3 H]carnitine

Each point represents the mean of results from two animals. The curves represent the fitted models derived from compartmental analysis. Blood plasma is regarded as the reservoir and the constants for the fitted disappearance curve are given in Table 2. The derived characteristics for the exchange of carnitine between blood plasma and the various tissues are presented in Table 3. (a) Blood plasma; (b) liver; (c) ●, kidney; ▲, muscle; (d) ●, spleen; ▲, brain; (e) ●, adrenal; ▲, lung; (f) ●, prostate; ▲, seminal vesicle; (g) ●, pancreas; ▲, testis; (h) ●, fat, ▲, heart.

Table 3. Results of fitting the models to the measurements of specific radioactivity in the tissues, expressed in terms of compartment size, turnover-time and flux of carnitine

The coefficients of the equation describing the behaviour of the specific radioactivity of carnitine in the blood plasma (Table 2) were used in the analysis. There was equal weighting of all data. Where possible the simpler, two-compartment model was used (that is, where the two-compartment model yielded a lower value for the data variance about the fitted line than did the three-compartment model). In cases where the three-compartment model provided a better description of the behaviour of the specific radioactivity in a tissue, the compartment exchanging carnitine more rapidly is listed first. Turnover time and flux are defined in the Materials and Methods section. Uncertainties are expressed as s.e.m. S^2 represents the variance of the data about the fitted line and $\bar{y}$ is the mean specific radioactivity of each tissue. The experimental points and the fitted lines are shown in Fig. 1.

	Compartment size (% of tissue carnitine)	Turnover time (h)	Carnitine flux (nmol/h per g fresh wt. of tissue)	S^2	$(S/\bar{y}) \times 100$
Kidney	100	0.415 ± 0.040	2240 ± 240	396	5.4
Spleen	69 ± 8	0.39 ± 0.22	654 ± 374	1092	10
	31 ± 8	7.8 ± 2.2	15.1 ± 5.6		
Liver	89 ± 1	1.31 ± 0.05	180 ± 22	158	3.6
	11 ± 1	903 ± 1980	0.032 ± 0.069		
Lung	30 ± 11	1.88 ± 0.72	88 ± 48	455	8.6
	70 ± 11	15.4 ± 4.7	25.1 ± 8.8		
Adrenal	89 ± 2	3.31 ± 0.14	72.3 ± 4.7	194	4.3
	11 ± 2	460 ± 720	0.07 ± 0.1		
Heart	100	20.7 ± 0.7	68.7 ± 3.0	162	8.0
Prostate	70 ± 2	2.92 ± 0.12	44.2 ± 5.6	88	3.4
	30 ± 2	84.0 ± 22.6	0.67 ± 0.20		
Seminal vesicle	45 ± 13	3.14 ± 0.79	35.4 ± 13.3	544	10
	55 ± 13	23.3 ± 10.3	5.76 ± 2.88		
Pancreas	57 ± 28	4.1 ± 1.2	25 ± 14	483	8.3
	43 ± 28	17 ± 12	4.5 ± 4.4		
Quadriceps muscle	18 ± 8	13.4 ± 4.7	14.0 ± 7.7	48	7.3
	82 ± 8	105 ± 18	8.0 ± 1.6		
Testis	47 ± 8	9.65 ± 2.13	9.43 ± 2.66	386	14
	53 ± 8	263 ± 150	0.38 ± 0.23		
Epididymal fat	100	5.20 ± 0.64	4.42 ± 0.67	533	8.0
Brain	10 ± 3	6.6 ± 2.6	1.8 ± 0.9	90	13
	90 ± 3	223 ± 34	0.47 ± 0.08		

Table 4. *Turnover of carnitine by the whole animal*

The total rate of excretion of carnitine was estimated in two of the animals by application of the Stewart–Hamilton equation (Shipley & Clark, 1972) to measurements of specific radioactivity in the urine, and also by applying the same analysis to the mean values of specific radioactivity in the blood plasma (see the Materials and Methods section). The rates of excretion of carnitine in the urine of two rats (and in a third rat, for which the above analysis could not be made because the urine collections were incomplete) were measured directly by enzyme assay. Uncertainties are expressed as $\pm$ S.E.M.

Time after injections (days)	Specific radioactivity of carnitine in each 24h urine collection (d.p.m./nmol)		
	Animal 1	Animal 2	Animal 3
1	—	925	939
2	1377	202	265
3	949	152	183
4	863	132	150
5	888	122	133
6	793	115	109
7	711	107	105
8	536	99	98
9	596	93	90
10	473	89	90
11	555	85	89
12	575	88	80
13	256	—	80
14	—	—	75
15	408	—	71
16	—	—	85
Labelled carnitine injected (μ Ci)	93.3	18.7	18.7
Body wt. (g)	350	390	400
Estimated total rate of excretion by analysis of urine (μ mol/day)	—	11.6 ± 1.1	9.08 ± 1.61
Measured urinary rate of excretion (μ mol/day)	2.82 ± 0.31	3.93 ± 0.29	2.80 ± 0.36
Urinary excretion as fraction of total	—	0.34	0.31
Estimate of total rate of excretion by analysis of specific radioactivity of blood plasma = $10.5 \pm 3.9 \mu$ mol/day			

flux and this caused large uncertainties to occur in the estimates of their turnover times. Nevertheless, it was quite clear from application of the criterion formulated by Zilversmit *et al.* (1943), as used in the analysis (McIntosh & Lutwak-Mann, 1972) that slowly exchanging pools of carnitine do exist in these tissues.

Among those tissues that were more accurately described by the three-compartment model, three groups are evident. One group, comprising the liver, spleen, adrenal and prostate, contained a rapidly exchanging compartment that was more than twice the size of the slowly exchanging compartment. The pancreas, seminal vesicle and testis represent a group in which the fast and slow compartments were of approximately the same size. Lung, skeletal muscle and brain, on the other hand, contained a rapidly exchanging compartment which was less than one-half the size of the slowly exchanging one.

Table 3 lists the tissues in order of decreasing flux of carnitine into or out of the tissue. With the three-compartment model, the total tissue flux is obtained by summing the fluxes for both tissue compartments. The derivation of total tissue flux depends on the

relative contribution of the two tissue compartments as well as on their total content of carnitine. Thus the flux of carnitine to the brain is low because not only has the brain a low content of carnitine but also most of this is contained in a compartment that has a slow rate of turnover. Tissues such as the kidney and spleen represent the opposite condition. Heart and skeletal muscle, on the other hand, despite having a very high content of carnitine display a relatively low flux owing to the fact that this carnitine exchanges quite slowly.

Total body turnover of carnitine

The pattern of specific radioactivity of carnitine in daily 24h collections of urine is shown in Table 4. These data, as well as the measurements of specific radioactivity in blood plasma, were used to derive estimates of the total excretion rate of carnitine, which are also listed in Table 4. The mean estimated total excretion of carnitine was 10.4μ mol/day, of which about 30% was in the urine.

Discussion

Carnitine content of the tissues

The amounts of total tissue carnitine found in the present work are similar to those reported in previous studies (Broekhuysen *et al.*, 1965; Marquis & Fritz, 1965a; Pearson & Tubbs, 1967). It is evident, however, that tissue concentrations of carnitine can be markedly influenced by diet, physiological status and environmental temperature (Strength *et al.*, 1965; Therriault & Mehlman, 1965a,b; Pearson & Tubbs, 1967; Mehlman *et al.*, 1969, 1971; Snoswell & Henderson, 1970). Heart and skeletal muscle contain the greatest concentrations of total carnitine, whereas fat and blood plasma have the least. High contents of carnitine in the adrenal gland have been reported by Broekhuysen *et al.* (1965) and Abdel-Kader & Wolf (1965), but could not be confirmed by Pearson & Tubbs (1967). In the present study we were also unable to confirm the existence of high contents of carnitine in the adrenal.

Compartmental analysis

Previous work has indicated that after the administration of radioactive carnitine, there is a substantial uptake by heart and skeletal muscle but only a small uptake by the brain (Yue & Fritz, 1960, 1962; S. Lindstedt & G. Lindstedt, 1961; Mehlman & Wolf, 1962). These studies did not however, relate the amount of tissue uptake to the tissue content, that is specific radioactivities were not determined. The present results are in general agreement with these previous experiments with respect to the extent of uptake of radioactivity by the different tissues. However, the determination of specific radioactivities coupled with compartmental analysis has enabled a more thorough appraisal to be made. Thus it is evident that all tissues finally come to equilibrium with the blood plasma by exchange of carnitine. There is, nonetheless, a wide variation between tissues in the ease and speed with which they attain this equilibrium. The kidney, spleen and liver exchange carnitine with the blood plasma relatively rapidly, whereas heart and skeletal muscle do so slowly. Many of the tissues appear to contain at least two pools of carnitine and in cases such as the brain, where most of the carnitine is in a slowly exchanging pool, the time to reach equilibrium is quite protracted. The very large estimates of turnover times of several of the slowly exchanging compartments are outside the time-scale of the experiments, as is reflected in the unrealistic values of the uncertainties associated with the estimates. It is difficult to envisage a physical explanation for the very slow rate of turnover shown by, for instance, the small compartment of the liver. A more plausible alternative is that the synthesis of carnitine known to occur

in this organ (Haigler & Broquist, 1974) diluted the specific radioactivity and contravened the inherent assumption of steady state, thereby confusing the analysis.

It would, of course, be of great interest to know what comprises the different tissue compartments. Unfortunately, the data cannot provide information on this point. There are a number of possibilities, however. The existence of more than one compartment could reflect the presence of more than one cell type within the tissue, several compartments within the cytoplasm of any particular cell type, or even more than one type of subcellular organelle. For instance, Ramsay & Tubbs (1974) have shown the presence of large amounts of carnitine in heart mitochondria; in this example, however, exchange with cytoplasmic carnitine is rapid in comparison with the time-scale of the present experiments and would go undetected. Alternatively, the carnitine could be distributed between the cells and extracellular fluid or even between free carnitine and carnitine esters. This latter process would probably also be undetected here because of the rapidity of this interconversion.

Metabolism of carnitine to other compounds

There was no indication in this study of any radioactive compound other than carnitine in the tissues or the urine. This result has been found previously (Yue & Fritz, 1960, 1962; S. Lindstedt & G. Lindstedt, 1961; G. Lindstedt & S. Lindstedt, 1961; Wolf & Berger, 1961) and indicates that carnitine is usually largely not metabolized. However, under certain conditions such as in pregnancy, or when the diet is rich in amino acids, and in other types of physiological stress, there can be extensive decarboxylation of carnitine to form β -methylcholine, which is excreted in the urine (Therriault & Mehlman, 1965b; Khairallah & Wolf, 1967; Mehlman *et al.*, 1969, 1971).

Contribution to carnitine in urine from the male reproductive tract

Table 4 shows that the specific radioactivity of carnitine in the urine was not always represented by the smooth curve which might be expected if carnitine in urine merely represents a filtrate of blood plasma. In fact, large amounts of carnitine are present in the male genital-duct system (Marquis & Fritz, 1965b; Brooks *et al.*, 1974). It is also apparent that among sexually inactive males, as in this study, the contents of the ductus deferens and epididymis are voided into the urine (Lino *et al.*, 1967) as well as being released by spontaneous ejaculation (Kihlström, 1965; Orbach *et al.*, 1967). Thus in male rats carnitine will be added to the urine by both the kidneys and the genital

system. The results of Brooks *et al.* (1973) demonstrate that radioactive carnitine is taken up into the epididymal fluid from the bloodstream, but it is not until about 2 weeks after the administration of label that this radioactive carnitine is likely to be excreted into the urine. During this period there will, of course, be addition of unlabelled carnitine to the urine from the ductus deferens.

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References

- Abdel-Kader, M. M. & Wolf, G. (1965) in *Recent Research on Carnitine* (Wolf, G., ed.), pp. 147–156, Massachusetts Institute of Technology Press, Cambridge, Mass.
- Broekhuysen, J., Rozenblum, C., Ghislain, M. & Deltour, G. (1965) in *Recent Research on Carnitine* (Wolf, G., ed.), pp. 23–25, Massachusetts Institute of Technology Press, Cambridge, Mass.
- Brooks, D. E., Hamilton, D. W. & Mallek, A. H. (1973) *Biochem. Biophys. Res. Commun.* **52**, 1354–1360
- Brooks, D. E., Hamilton, D. W. & Mallek, A. H. (1974) *J. Reprod. Fert.* **36**, 141–160
- Haigler, H. T. & Broquist, H. P. (1974) *Biochem. Biophys. Res. Commun.* **56**, 676–681
- Khairallah, E. A. & Mehlmán, M. A. (1965) in *Recent Research on Carnitine* (Wolf, G., ed.), pp. 57–62, Massachusetts Institute of Technology Press, Cambridge, Mass.
- Khairallah, E. A. & Wolf, G. (1967) *J. Biol. Chem.* **242**, 32–39
- Kihlström, J. E. (1965) *Acta Physiol. Scand.* **65**, 61–64
- Lindstedt, G. & Lindstedt, S. (1961) *Biochem. Biophys. Res. Commun.* **6**, 319–323
- Lindstedt, S. & Lindstedt, G. (1961) *Acta Chem. Scand.* **15**, 701–702
- Lino, B. F., Braden, A. W. H., & Turnbull, K. E. (1967) *Nature (London)* **213**, 594–595
- Marquis, N. R. & Fritz, I. B. (1964) *J. Lipid Res.* **5**, 184–187
- Marquis, N. R. & Fritz, I. B. (1965a) *J. Biol. Chem.* **240**, 2193–2196
- Marquis, N. R. & Fritz, I. B. (1965b) *J. Biol. Chem.* **240**, 2197–2200
- McIntosh, J. E. A. & Lutwak-Mann, C. (1972) *Biochem. J.* **126**, 869–876
- Mehlman, M. A. & Wolf, G. (1962) *Arch. Biochem. Biophys.* **98**, 146–153
- Mehlman, M. A., Abdel-Kader, M. M. & Therriault, D. G. (1969) *Life Sci.* **8**, II, 465–472
- Mehlman, M. A., Therriault, D. G. & Tobin, R. B. (1971) *Metabolism* **20**, 100–107
- Orbach, J., Miller, M., Billimoria, A. & Solhkhah, N. (1967) *Brain Res.* **5**, 520–523
- Pearson, D. J. & Tubbs, P. K. (1964) *Biochim. Biophys. Acta* **84**, 772–773
- Pearson, D. J. & Tubbs, P. K. (1967) *Biochem. J.* **105**, 953–963
- Ramsay, R. R. & Tubbs, P. K. (1974) *Biochem. Soc. Trans.* **2**, 1285–1286
- Shipley, R. A. & Clark, R. E. (1972) *Tracer Methods for in vivo Kinetics*, Academic Press, London and New York
- Snoswell, A. M. & Henderson, G. D. (1970) *Biochem. J.* **119**, 59–65
- Strength, D. R., Yu, S. Y. & Davies, E. Y. (1965) in *Recent Research on Carnitine* (Wolf, G., ed.), pp. 45–56, Massachusetts Institute of Technology Press, Cambridge, Mass.
- Therriault, D. G. & Mehlmán, M. A. (1965a) in *Recent Research on Carnitine* (Wolf, G., ed.), pp. 141–145, Massachusetts Institute of Technology Press, Cambridge, Mass.
- Therriault, D. G. & Mehlmán, M. A. (1965b) *Can. J. Biochem.* **43**, 1437–1443
- Wolf, G. & Berger, C. R. A. (1961) *Arch. Biochem. Biophys.* **92**, 360–365
- Yue, T. N. & Fritz, I. B. (1960) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **19**, 223
- Yue, T. N. & Fritz, I. B. (1962) *Am. J. Physiol.* **202**, 122–128
- Zilversmit, D. B., Entenman, C. & Fishler, M. C. (1943) *J. Gen. Physiol.* **26**, 325–331