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ACTIVE TRANSPORT OF BUTYROBETAINE AND CARNITINE INTO ISOLATED LIVER CELLS

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SUMMARY

1. The liver cells lose the major part of their carnitine during the commonly used isolation procedure by the collagenase-perfusion method.

2. The cells take up carnitine and the carnitine precursor butyrobetaine when these substances are added to the medium. The carnitine content of isolated liver cells can increase to about 15 mM with no apparent harm to the cells.

3. The data indicate the existence of a common carrier in the plasma membrane which mediates the uphill transport of both carnitine and butyrobetaine. The carrier has a high affinity for butyrobetaine ($K_m = 0.5$ mM) and a lower one for carnitine ($K_m = 5.6$ mM).

4. The intracellular butyrobetaine is hydroxylated to carnitine with a rate of approximately 0.33 μmol · g wet weight^{-1} · h^{-1} which is sufficient to cover the turnover of carnitine in the whole rat. Carnitine is effectively esterified in the liver cells to acetylcarnitine and long-chain acylcarnitines.

5. Both carnitine and acetylcarnitine are released from the cells. The release of both compounds is probably physiological since it was found that acetylcarnitine constitutes a similar fraction of the total acid soluble carnitine both in the blood and liver of the intact rat.

INTRODUCTION

Isolated liver cells are widely used for metabolic studies, also for studies of fatty acid metabolism. However, especially for studies on fatty acid metabolism, it is important to note that isolated liver cells retain only about 20 % of the carnitine originally present in the intact liver. It has been suggested that changes in the rate of fatty acid oxidation and ketogenesis in the liver in different nutritional states result primarily from changes in the ability of the liver to transport activated fatty acids through the inner mitochondrial membrane [1, 2]. Both changes in the activity of carnitine palmityltransferase [3] and changes in carnitine content of the liver [4] have been suggested as regulatory factors. We have already reported that carnitine stimulates palmitate oxidation in isolated liver cells [5]. Variation in the carnitine content of isolated cells thus can be used as a tool to study mechanisms which regulate the flux

through the β-oxidation sequence in different metabolic states. For such studies it is important to know how uptake and release of carnitine in isolated liver cells is governed.

The last step in carnitine biosynthesis-hydroxylation of butyrobetaine, is localized mainly in the liver and the synthesized carnitine is subsequently transferred to other tissues [6–8]. Therefore we have studied: (1) the transport of both carnitine and butyrobetaine through the plasma membrane of isolated liver cells, (2) the hydroxylation of butyrobetaine to carnitine, (3) the esterification of intracellular carnitine, (4) the subsequent release of carnitine and acetylcarnitine to the medium.

MATERIALS AND METHODS

Chemicals

(−)- and (+)-carnitine were a gift from Otsuka Pharmaceutical Company. Osaka, Japan. Butyrobetaine, (−)-norcarnitine, [*Me*-^{3}H]butyrobetaine (spec. act. 80 Ci/mol), [*Me*-^{3}H]-(−)carnitine (spec. act. 80 Ci/mol) and [*Me*-^{3}H](+)-carnitine (spec. act. 50 Ci/mol) were synthesized as previously described [9–11]. 1-Dimethylamine, 2-propanol and methylcholine were prepared from 1-amino 2-propanol by a methylation procedure as described for the synthesis of butyrobetaine [9]. ^3H_2O, [*carboxy*-^{14}C]inulin and acetyl-CoA (acetyl-1^{14}C)were from NEN Chemicals GmbH, G.F.R. Carnitine acetyltransferase was obtained from Sigma, St. Louis, U.S.A. Silicone oil AR 100 was obtained from Wacker Chemie AG (München, G.F.R.). Other reagents were commercially available products of analytical grade.

Isolation of liver cells

The hepatocytes were prepared from the liver of male Wistar rats which had free access to the standard pellet diet and water until they were killed. The parenchymal liver cells were prepared and purified according to Seglen [12] except that only 0.03 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) was used in the suspension medium. The cell suspensions were always examined for trypan blue exclusion. A high percentage of unstained cells (over 95 %) was routinely observed. A lack of stimulation by succinate of the cellular respiration repeatedly observed, was taken as a criterion of the intactness of the plasma membrane.

Incubation conditions and the separation of the cells from the medium

Cells were incubated in oxygenated suspension medium in plastic reagent tubes (0.2–0.4 ml incubation volume) for shorter incubation periods, and in 25 ml erlenmeyer flasks (2–4 ml incubation volume) for longer incubation periods. Cells were separated from the medium by two methods. (1) centrifugal filtration through the silicone oil AR 100 (13). ^3H_2O and [*carboxy*-^{14}C]inulin were used for determination of cellular water space and the extracellular volume of adherent fluid. The cell water varied between 2.7–3.0 μl/mg protein and the volume of extracellular adherent fluid was 0.9–1 μl/mg protein. (2) The process of uptake was stopped by cooling down the incubation mixture on ice and the cells were separated from the incubation medium by centrifugation for 5 min at 250 × *g*. The cell suspensions were subsequently washed twice with 5 ml cold suspension buffer. Table I shows that the cells retained their carnitine when kept at 0 °C. Two washings removed completely all the extracellular

TABLE I

EFFECT OF WASHING AT 0 °C AND CENTRIFUGATION THROUGH THE SILICONE OIL ON THE RETENTION OF CARNITINE BY THE LIVER CELLS

The cells (26 mg protein) were incubated with different concentrations of [^{3}H]carnitine for 45 min in total volume of 3 ml. The incubation mixture was divided in portions and washed the indicated number of times with 5 ml cold, carnitine free suspension buffer. Between washings the cells were centrifuged for 5 min at 250 × *g*. 0.1 ml samples of the incubation mixture were centrifuged through the silicone oil.

Added carnitine (mM)	Number of washings	Carnitine retained by the cells (nmol · μl cell water^{-1})	
		Washing of the cells	Centrifugation through the silicone oil
0.05	0	0.280	
	1	0.200	
	2	0.196	
	6	0.192	0.201
5	0	21.30	
	1	8.67	
	2	7.70	
	6	7.65	7.95
Freezing and thawing of the cells (2 times)		0.38	–

carnitine. Both methods gave virtually identical results in the present transport studies. Freezing and thawing of the cells led to a lost of 95 % of intracellular carnitine (Table I).

Chromatographic technique

5 % trichloroacetic acid extracts of the cells and supernatant were used for chromatography. The trichloroacetic acid was removed by extraction 3 times with 5 volumes of diethyl ether. The remaining aqueous extract was blown to dryness under a current of air; the sediment was dissolved in a small volume of water/methanol (1 : 1) and chromatographed on thin-layer plates (Stahl H). For separation of butyrobetaine and carnitine+acetylcarnitine, methanol/acetone/concentrated HCl (9 : 1 : 1, v/v/v) were used as a developing solvent. R_F values for butyrobetaine and carnitine+acetylcarnitine were respectively 0.35 and 0.50. For separation of acetylcarnitine from butyrobetaine+carnitine, methanol/chloroform/H_2O/concentrated NH_3/concentrated HCOOH (55 : 50 : 10 : 7.5 : 2.5, v/v/v/v/v) were used and R_F values were respectively 0.25 and 0.17.

Analytical methods

Carnitine was determined according to Cederblad and Lindstedt [14], except that only HEPES buffer was always used in the reaction mixture. It is worth while to notice that other commonly used buffers like TES (*N*-Tris-(hydroxymethyl)-methyl-2-aminoethane-sulfonic acid), Tris-(hydroxymethyl)aminomethan, and especially TRICINE (*N*-Tris-(hydroxymethyl)methyl glycine) undergo enzymatic acetylation by acetyl-CoA in the presence of carnitine acetyltransferase, and therefore can not

be used in the incubation mixture (Bremer, J. and Christiansen, R. Z., unpublished results).

The cellular protein was determined by a method of Lowry et al. [15]. Long-chain acylcarnitines were measured as the butanol-extractible radioactivity after incubation of the cells in the presence of [^{3}H](−)-carnitine.

RESULTS

Carnitine content of isolated liver cells

During the isolation of liver cells the carnitine is gradually released to the medium (Table II). About half of the carnitine content of the liver was lost during the primary perfusion. The initial cell suspension had about one-third of the carnitine concentration of the liver. The subsequent incubation of the cells, filtration and centrifugation (see Material and Methods) reduced the carnitine content further and resulted in a preparation of purified parenchymal cells which contained about 0.1 nmol · μl intracelllular water^{-1} of carnitine.

Table II shows that two methods used to follow the release of carnitine during the isolation procedure of liver cells gave nearly identical results. One should notice, that the values describing the carnitine concentration of the liver or isolated cells vary depending on whether μmol of carnitine are related to g wet w. or ml intracellular water. Thus the carnitine concentration for example in the liver would be approximately 0.29 μmol · g wet w.$^{-1}$ (assuming that the protein constitutes about 20 % of wet w. of the liver of fed rat) and 0.49 μmol · ml intracellular water^{-1}.

TABLE II

DECREASE IN CARNITINE CONTENT DURING LIVER CELLS PREPARATION

A, carnitine was estimated according to Cederblad and Lindstedt [14]. Mean ±S.D. were calculated from 9 experiments. B, [^{3}H]butyrobetaine (0.1 mC; specific radioactivity 80 Ci/mol) was injected to the rats 24 h before the isolation of hepatocytes. Mean ±S.D. were calculated from 5 experiments. Cells I, initial cell suspension; cells II, final suspension of parenchymal cells.

Fraction	A		B
	nmol · mg protein^{-1} (mean ±S.D.)	%	cpm · mg protein^{-1} % (mean ±S.D.)
Liver	1.46±0.16	100	100
Liver after perfusion	0.80±0.18	54.8	56.3± 9.0
Cells I	0.50±0.24	34.2	35.2±10.2
Cells II	0.32±0.07	21.9	23.1± 2.2

Active transport of carnitine and butyrobetaine

Both carnitine and butyrobetaine are transported into the cells against a concentration gradient. Fig. 1 presents the time curves of the transport. The results are based on the total radioactivity which entered the cells. Enzymatic measurements of total carnitine content in the cells confirmed that there is a net uptake of carnitine in agreement with the results obtained with radioactive substrate (not shown). The approximate steady state of the transport was reached in about 90 min with all the

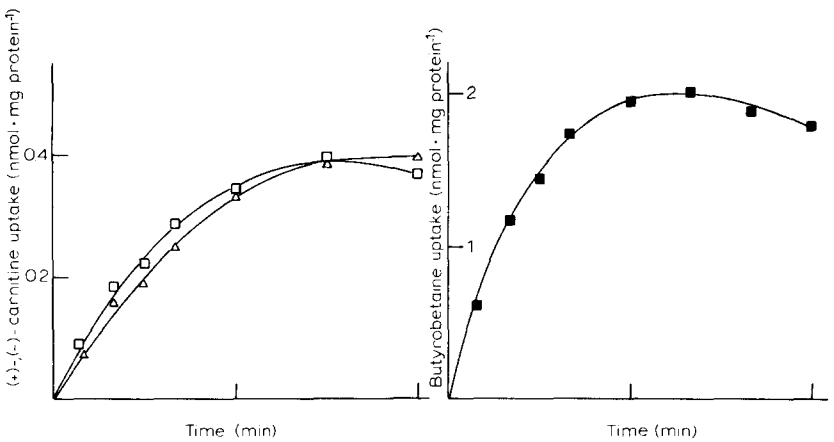

Fig. 1. The time course of (−)- and (+)-carnitine and butyrobetaine transport into isolated liver cells. The liver cells (30,1 mg protein) were incubated in total volume of 4 ml with radioactive (−)-carnitine (□—□), (+)-carnitine (△—△), and butyrobetaine (■—■) in the concentration of 0.025 mM. At indicated times 0.1 ml samples were centrifuged through the silicone oil. The radioactivity in the supernatant and perchloric acid extracts of the pellet was measured. The amount of carnitine and butyrobetaine were calculated on the basis of specific radioactivity. No correction was made for hydroxylation of butyrobetaine and esterification of carnitine.

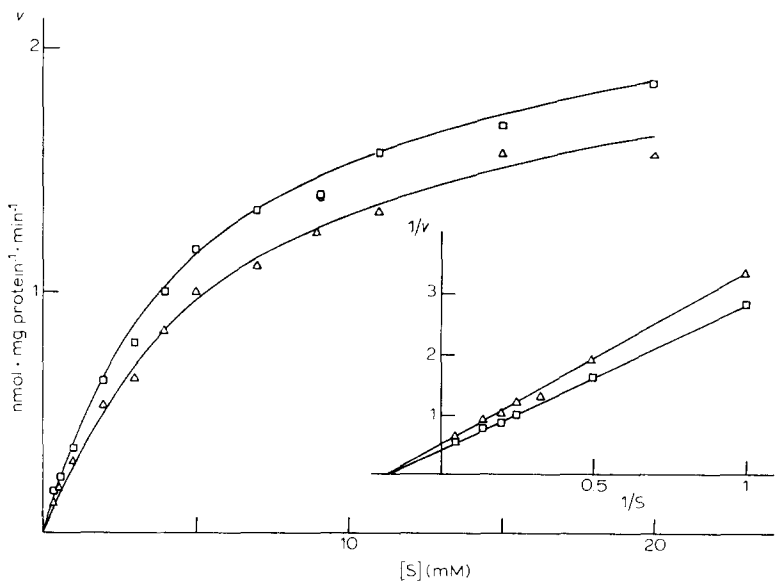

Fig. 2. The concentration curve for (−)- and (+)-carnitine transport into isolated liver cells. The cells (approx. 1 mg protein) were incubated in a volume of 0.2 ml for 5 min at 37 °C. with different concentrations of radioactive (+)- or (−)-carnitine. The reaction was stopped by centrifugation of the cells through the silicone oil. (□—□), (−)-carnitine; (△—△), (+)-carnitine. The kinetic data were as follows: for L-carnitine K_m = 5.6 mM, V = 2.4 nmol · mg protein^{-1} · min^{-1}, for D-carnitine K_m = 5.6 mM, V = 2 nmol · mg protein^{-1} · min^{-1}.

three substrates (Fig. 1) and the calculated concentration gradient (disregarding the formation of metabolites) between cells and medium was below 10 for (−)- and (+)-carnitine, and almost 80 for butyrobetaine. (−)-Carnitine entry is slightly more rapid than that of (+)-carnitine.

The transport of (+)- and (−)-carnitine shows saturability (Fig. 2). V for (+)-carnitine transport is only slightly lower than for (−)-carnitine and the K_m values for the two isomers are about the same. Thus there is no or only a weak stereospecificity of the transport.

The concentration curve for butyrobetaine uptake (Fig. 3) seems to be the sum of two different transport systems: one with high affinity for the substrate (K_m = 0.5 mM) and the other with very low affinity (K_m = 30 mM). The latter value can probably be considered as nonspecific for butyrobetaine. In further studies we paid attention only to the first transport system and the results were corrected for the amount of butyrobetaine which could be transported by the second system with a given concentration of butyrobetaine in the medium.

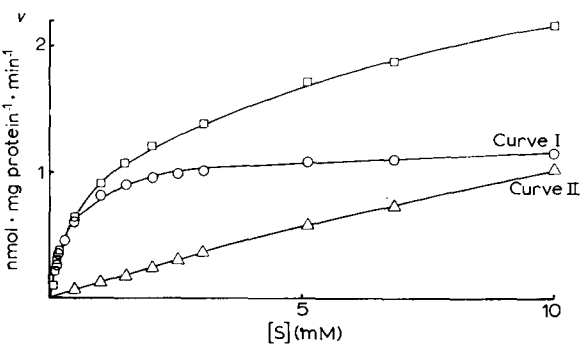

Fig. 3. The concentration curve for butyrobetaine transport into isolated liver cells. The incubations conditions were as described in the legend to Fig. 2. (□−□), experimental data; (○−○), the theoretical curve I calculated on the assumption that with low concentrations of butyrobetaine in the medium the second transport system does not interfere, (△−△), the theoretical curve II obtained by subtraction of the values of curve I from experimental data. The values of the curve I were corrected back for the interference of the system II, after kinetic data for system II have been calculated. The kinetic data were calculated from Lineweaver-Burk plots and were as follow: system I K_m = 0.5 mM, V = 1.2 nmol · mg · protein^{-1} · min^{-1}, system II K_m = 30 mM. V = 4 nmol · mg protein^{-1} · min^{-1}.

Fig. 4 shows that 2,4-dinitrophenol has a strong inhibitory effect on the transport. With 0.5 mM 2,4-dinitrophenol in the medium the concentration gradient between cells and medium decreased from 10.4 to 1.8 and from 3.3 to 1.4 respectively for butyrobetaine and carnitine. One can notice that only 0.1 mM 2,4-dinitrophenol is needed to uncouple the mitochondrial respiration. However, the uncoupler is probably bound to cytoplasmic protein and other components in the cells and therefore a higher concentration has to be used to get the uncoupling effect in the cells. We have also observed that 0.3–0.4 mM dinitrophenol is needed to give maximal stimulation of endogenous respiration of the liver cells. Ouabain, which is known to inhibit the sodium potassium pump, did not affect the transport in the concentration of 0.01–0.03 mM (not shown).

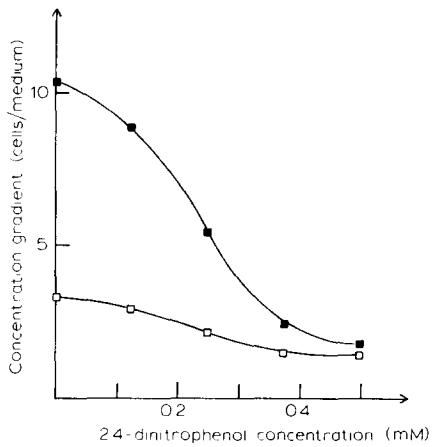

Fig. 4. The effect of 2,4-dinitrophenol on the transport of L-carnitine and butyrobetaine into the isolated liver cells. The cells (4.4 mg protein) were incubated in a volume of 0.4 ml for 20 min at 37 °C with radioactive 0.5 mM butyrobetaine and 0.5 mM carnitine without and with the different concentrations of 2,4-dinitrophenol. The incubation was stopped by centrifugation of the cells through the silicone oil. (■—■), butyrobetaine; (□—□), (—)-carnitine.

Fig. 5 presents the Arrhenius plot for carnitine transport which was linear between 15 and 40 °C. A relatively high activation energy of 15.6 kcal indicates the existence of a carrier for carnitine transport. Below 15 °C the activation energy is much lower. The exact value was not determined because of slow transport and the low accuracy of the experimental values in this temperature range. It seems probable that at low temperature a simple diffusion process prevails.

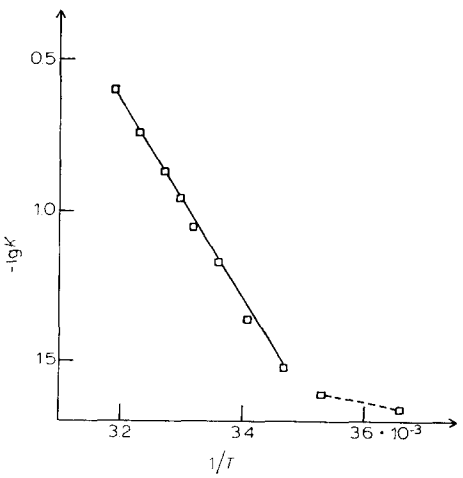

Fig. 5. The effect of the temperature on the rate of (—)-cartinine transport into isolated liver cells (Arrhenius plot). The incubation conditions were as described in the legends to Fig. 2. Reaction rate is expressed as nmol · protein^{-1} · min^{-1}. The activation energy was calculated to be equal to 15.6 kcal between 15 and 40 °C (mean from 4 experiments).

TABLE III

SOME KINETIC CONSTANTS FOR BUTYROBETAINE AND CARNITINE TRANSPORT

The incubation conditions were as described in the legend to Fig. 2. The values were calculated from Lineweaver-Burk plots. The data for butyrobetaine were corrected for the interference of the transport system II.

Inhibitor	Butyrobetaine transport			Inhibitor	Carnitine transport		
	Apparent K_m (mM)	V (nmol · min^{-1} · mg protein^{-1})	K_i for carnitine (mM)		Apparent K_m (mM)	V (nmol · min^{-1} · mg protein^{-1})	K_i for butyrobetaine (mM)
None	0.5	1.2	–	None	5.6	2.4	–
(−)-carnitine (4 mM)	0.8	1.2	6.6	butyrobetaine (1 mM)	12.5	2.4	0.8
(−)-carnitine (10 mM)	1.1	1.2	7.6	butyrobetaine (4 mM)	28.6	2.4	1.0

The high activation energy and the saturability of the transport indicates that there exists a carrier for carnitine and butyrobetaine transport. The uptake of carnitine and butyrobetaine against a concentration gradient and the inhibition by an uncoupler (2,4-dinitrophenol) indicates the use of energy for the transport processes.

One or two carriers?

The next problem to be solved is if carnitine and butyrobetaine share the same transport agency or if they are transported independently of each other. Both of them behave as mutual competitive inhibitors in the trasport. Table III shows that the respective K_m and K_i values describing the interaction between carnitine and butyrobetaine transport are at least in the same range of values and are consistent with each other. These results show that both substances most likely have one common carrier.

The inhibitory effects of some structural analogs on the transport of carnitine and butyrobetaine were studied (Table IV). All of them were competitive inhibitors and the calculated K_i values were very similar for inhibition of both carnitine and betaine transport. Again it can be concluded, that carnitine and butyrobetaine are transported by the same carrier. It is not clear if nor-carnitine, choline, 1-dimethylamine-2-propanol and methylcholine are also transported or only inhibit the transport without being taken up by themselves. In order to check this point the radioactive compounds have to be synthesized.

TABLE IV

THE INHIBITION OF CARNITINE AND BUTYROBETAINE TRANSPORT BY STRUCTURALLY RELATED COMPOUNDS

The incubation conditions were as described in the legend to Fig. 2. K_I values were calculated from Lineweaver-Burk plots.

Inhibitor	K_I (mM)	
	Carnitine transport	Butyrobetaine transport
Nor-carnitine	4	5
Choline	20	18
1-Dimethylamine 2-propanol	16	14
Methylcholine	49	46

Esterification of carnitine in the cells

Intracellular carnitine is efficiently esterified to acetylcarnitine and long-chain acylcarnitines (Table V). The acetylcarnitine-carnitine ratio is kept quite stable and decreases only from 0.78 to 0.41 while the intracellular total carnitine concentration increases over 250 times. This result indicates that there is a very active regulation of the supply of acetyl groups in the cell. The carnitine acetyltransferase reaction, which can be considered as a "sink" for acetyl groups, evidently did not influence the acetyl-CoA/CoA ratio of the mitochondria to any great extent. Long-chain acylcarnitines also increased with increasing intracellular concentrations of carnitine, but not proportionally and the ratio long-chain acylcarnitine/carnitine decreased significantly.

TABLE V

THE TRANSPORT AND ESTERIFICATION OF (−)-CARNITINE IN ISOLATED LIVER CELLS

The cells (18 mg protein) were incubated in the volume of 2 ml for 2 h with different concentrations of [^{3}H]carnitine. The reaction was stopped by centrifugation of the cells through the silicone oil. The separation of acetylcarnitine and carnitine and measurement of long-chain acylcarnitines were performed as described in Materials and Methods.

Carnitine added (mM)	Intracellular concentration (nmol · mg protein^{-1})				Gradient cells/medium	
	Total carnitine	Acetylcarnitine	Long-chain acylcarnitines	Free carnitine	Free carnitine	Total carnitine
0.01	0.19	0.07	0.03	0.09	3.0	7.9
0.05	0.91	0.34	0.09	0.48	3.2	7.5
0.5	6.72	2.44	0.27	4.01	2.7	5.3
2	21.80	7.12	0.61	14.07	2.3	4.2
5	48.30	13.75	1.11	33.44	2.2	3.6

The release of carnitine and acetylcarnitine from the cells

In the intact animal butyrobetaine is hydroxylated to carnitine mainly in the liver and carnitine is subsequently transported from the liver to other tissues. [6–8] In the isolated cells the maximal concentration of "unchanged" butyrobetaine is reached within one hour and then decreases again, while carnitine and acetylcarnitine concentrations increase (Fig. 6). However, the cells keep a very high gradient of butyrobetaine which decreases only insignificantly during the incubation period. Both carnitine and acetylcarnitine were released from the cells after a lag period. Acetylcarnitine seemed to be preferentially released from the cells. It may be due to a difference in the K_m values for efflux between carnitine and acetylcarnitine. Fig. 6 and Table VI show that the ratio acetylcarnitine/carnitine in the cells varied around the value of 0.9 when carnitine was continuously generated from butyrobetaine. Butyrobetaine was linearly hydroxylated to carnitine (Fig. 6) with an observed maximal rate of 1.5 nmol · mg protein^{-1} · h^{-1} with 0.57 mM butyrobetaine in the cells. Butyrobetaine hydroxylase is apparently inhibited by the excess of the substrate, as the total amount of carnitine and acetylcarnitine produced from butyrobetaine decreases with increasing concentrations of intracellular byturobetaine (Table VI).

The release of acetylcarnitine from the cells seems to correspond to the situation in vivo. In a rat with its carnitine pool labelled by the injection of [Me-^{3}H] butyrobetaine, acetylcarnitine was found both in the liver and a blood plasma (Fig. 7).

The maximal acetylcarnitine/carnitine ratio was observed after 3 h after butyrobetaine injection both in the blood and liver. Thereafter the percentage of radioactivity found in acetylcarnitine decreased and reached the constant value of approximately 30 and 38 % in plasma and liver respectively after 6 h. After 1 h the

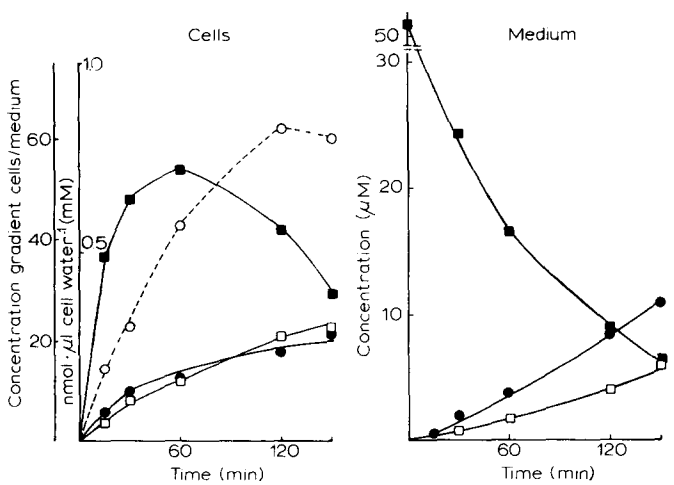

Fig. 6. The transport and turnover of butyrobetaine in isolated liver cells. The cells (36 mg protein) were incubated in total volume of 4 ml with radioactive 0.051 mM butyrobetaine. At indicated times 0.5 ml samples were spinned down and washed as described in Materials and Methods. Both medium and trichloroacetic acid extract of the cells were chromatographed on silica gel plates in order to separate carnitine+butyrobetaine from acetylcarnitine and carnitine+acetylcarnitine from butyrobetaine. (■—■), butyrobetaine; (●—●), acetylcarnitine; (□—□), cartnitine; (○—○), the concentration gradient of butyrobetaine between cells and medium.

TABLE VI

THE TRANSPORT AND TURNOVER OF BUTYROBETAINE IN PARENCHYMAL LIVER CELLS

The cells (36 mg protein) were incubated in a total volume of 4 ml with different concentrations of radioactive butyrobetaine for 2 h. Incubations were as described in the legend to the Fig. 6.

Butyrobetaine added (mM)	Intracellular concentration (nmol · mg protein^{-1})				Concentration gradient of butyrobetaine cells/medium	Total hydroxylated butyrobetaine* (nmol · mg protein)
	Total butyrobetaine transported	Butyrobetaine "unchanged"	Carnitine*	Acetylcarnitine*		
0.017	1.01	0.27	0.36	0.38	67.7	1.48
0.051	3.27	1.7	0.84	0.73	62.0	2.98
0.254	16.94	15.58	0.65	0.71	58.0	2.77
0.509	26.69	25.61	0.64	0.45	33	2.33
1.018	32.0	31.35	0.40	0.25	14.5	1.87

* The results represent only carnitine and acetylcarnitine which were produced from butyrobetaine.

** The results represent the sum of acetylcarnite and carnite both in the cells and in the medium.

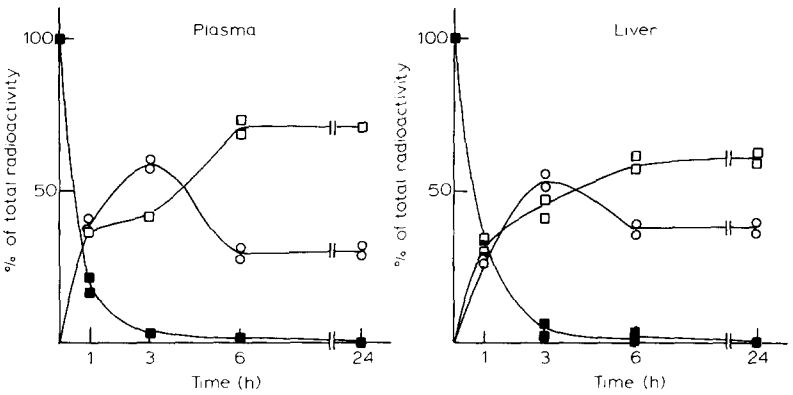

Fig. 7. The metabolism of butyrobetaine and carnitine in the rat in vivo. 0.4 mC [*Me*-^{3}H]butyrobetaine (specific radioactivity 80 Ci/mol) was given intraperitoneally to the rats. At indicated time intervals the rats were anesthetized and the blood and liver samples were removed. The livers were immediately freeze-clumped at the temperature of liquid air, homogenized in 5 % trichloroacetic acid and centrifuged to remove the protein. Blood was collected from the abdominal aorta into heparinized syringes. After removal of the erythrocytes by centrifugation the plasma proteins were precipitated with trichloroacetic acid (final concentration of 5 %). Both liver and plasma supernatants were extracted with diethyl ether to remove trichloroacetic acid and chromatographed on silica gel plates as described in Materials and Methods. The values at each time point are from two different animals (■—■), butyrobetaine; (□—□), carnitine; (○—○), acetylcarnitine.

radioactivity was approximately 19 and 33 % of free butyrobetaine in the plasma and liver respectively, which probably reflects the active transport of butyrobetaine into liver cells.

Since in vivo studies have shown that butyrobetaine is converted to carnitine almost exclusively in the liver of the rat [6–8], the initial high acetylcarnitine/carnitine ratio in the plasma (Fig. 7) strongly indicates that the acetylcarnitine had been released by the liver. The preference in releasing acetylcarnitine compared to free carnitine can be noticed. This finding may suggest that the transport of carnitine from the liver to other tissues, at least in part, takes place in the form of acetylcarnitine.

The decline in the cellular [^{3}H]carnitine and [^{3}H]butyrobetaine content by the release into 90 volumes of carnitine/butyrobetaine free medium was studied. Fig. 8 shows, that the exodus curve for butyrobetaine flattens much earlier than the exodus curve for carnitine. This flattening is probably due to the low K_m value for butyrobetaine reentry. After 90 min of the incubation there is 0.028 mM butyrobetaine in the medium, which corresponds to the reentry rate of 0.069 nmol · mg protein^{-1} · min^{-1}. The concentration gradient is about 100, which is close to that observed in the uptake studies (Fig. 1, Table VI). One should notice that what we consider as butyrobetaine on the basis of radioactivity, is in reality the mixture of butyrobetaine, carnitine and acetylcarnitine. However, with the high content of butyrobetaine in the cells, carnitine and acetylcarnitine would constitute only a small percentage of total radioactivity.

After 90 min of incubation of the carnitine-preloaded cells there is 0.062 mM carnitine in the medium, which corresponds to the reentry rate of 0.026 nmol · mg protein^{-1} · min^{-1}, which is only 38 % of that for butyrobetaine. The concentration gradient of carnitine is about 40 within 90 min and is still decreasing. The differences

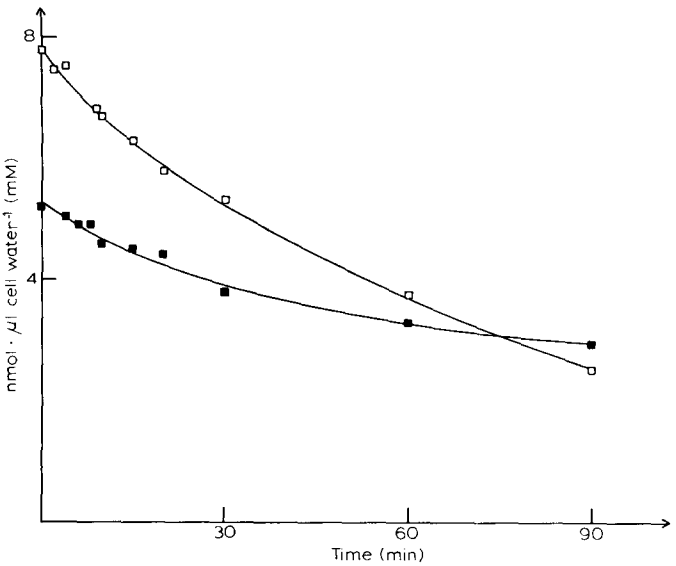

Fig. 8. The exodus of radioactive (−)-carnitine and butyrobetaine from liver cells at 37 °C. Liver cells (29 mg protein) were preincubated for 1 h in the total volume of 4 ml with radioactive 0.25 mM butyrobetaine and 2.5 mM carnitine, washed with the cold buffer from extracellular carnitine or butyrobetaine, suspended in 95 volumes (5 ml) of carnitine/butyrobetaine free medium and incubated at 37 °C. At different times 0.1 ml samples were centrifuged through the silicone oil and the radioactivity in the supernatant and pellet was measured (□−□), (−)-carnitine; (■−■), butyrobetaine.

in the K_m values for uptake can probably explain why liver cells keep a very high concentration gradient of butyrobetaine and a much lower one of carnitines (Tables V and VI).

DISCUSSION

The presented results show that carnitine and butyrobetaine are transported through the plasma membrane of isolated liver cells against a concentration gradient and that the transport is probably mediated by a common carrier, which has 10-times higher affinity for butyrobetaine than for carnitine.

Bøhmer et al. (Bøhmer, T. and Eiklid, K., unpublished results) have found, that carnitine is actively taken up by human heart cells in culture. The K_m value found for carnitine transport was in the range of a few μM. It is not certain if results obtained on tissue culture reflect the situation in vivo especially as it was reported that perfused rat heart showed only a very limited uptake of carnitine [16]. However, the big difference in K_m values for carnitine transport in rat liver cells and human heart cells may also be due to a different function of these two organs in carnitine metabolism. The blood plasma with its low carnitine concentration (0.05 mM)[17, 18] is the source of carnitine for the heart, while liver supplies the whole body with carnitine. Only the liver (and to some extent testis) can hydroxylate butyrobetaine to carnitine, which is subsequently released to the blood and redistributed to other tissues [6–8]. In our experiments butyrobetaine was in fact transported into the liver cells, hydrox-

ylated to carnitine and subsequently acylated and/or released. Unexpectedly both carnitine and acetylcarnitine were released from the cells. This finding evidently reflects the situation in vivo as we have found that acetylcarnitine constitutes a similar fraction of the total acid soluble carnitine both in the blood and in the liver of the intact rat. In contrast Maebashi et al. [19] did not find any carnitine esters in human serum. So far, we have not studied the uptake of acetylcarnitine in liver or other tissues, but its release from liver cells indicates that it may be transported in the blood and taken up by the other tissues. Butyrobetaine was hydroxylated to carnitine with an observed maximal rate of 1.5 nmol · mg protein^{-1} · h^{-1} by the intact liver cells. It corresponds to approximately 0.3 μmol · g wet w.$^{-1}$ · h^{-1} compared to a value of 1 μmol carnitine formed/g of rat liver per h, which was reported by Lindstedt [6] for butyrobetaine hydroxylation by a partially purified soluble protein fraction from rat liver under optimal conditions in vitro. From data given by Khairallah and Mehlman [20], the daily formation of carnitine is approximately 30 μmol in a 200 g rat or 0.15 μmol/g of rat liver per h. Thus the observed butyrobetaine hydroxylation rate of the liver cells is still sufficient for the estimated rate of carnitine synthesis in the whole rat.

An increase in the intracellular concentration of carnitine is accompanied by increase in long-chain acylcarnitine and acetylcarnitine concentration, which is probably the reflection of enhanced oxidation of fatty acids. Acetylcarnitine increases almost proportionally to the concentration of intracellular carnitine even if it is as high as 16 mM. On the other hand, we have found that approximately 0.5 mM carnitine in the cells gives the maximal stimulation of ketone bodies' production from added palmitate (not shown). Therefore it seems probable that when cells are overloaded with carnitine relatively more acetyl-CoA reacts to give acetylcarnitine and less is directed to ketone bodies' production. Mc Garry et al. [4] observed that addition of carnitine to the medium perfusing liver from fed rats led to the significant acceleration in the ketone bodies' production from 0.7 mM oleic acid. We have shown that in isolated cells carnitine stimulates the oxidation of palmitate (mainly to ketone bodies) and that this effect is especially strong when the cells from refed rats are used [5]. The present results have shown that it is possible to vary the intracellular concentration of carnitine by incubation of the cells with added carnitine. The uptake of carnitine has a much higher maximum rate than the conversion of butyrobetaine to carnitine, which also increases the intracellular carnitine concentration. The rapid transport of carnitine into the isolated liver cells makes it possible to study the effect of carnitine and with optimal carnitine concentration the effects of other factors, which may regulate hepatic fatty acid metabolism in different nutritional and metabolic states.

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