erschien reizvoll zu untersuchen, ob die alkalische Hydrolyse des *N,N,N',N'*-Tetramethyl-*S*-(2-phenyläthyl)-thiouroniumhydroxids nicht analog dem Hofmann-Abbau Styrol, Wasser und *N,N,N',N'*-Tetramethylthioharnstoff liefern würde. Dies ist nicht der Fall; man erhält lediglich 2-Phenyläthylmerkaptan und *N,N,N',N'*-Tetramethylharnstoff.

Die pharmakologische Prüfung der Verbindungen zeigte keine Wirkungen von praktischem Interesse. Nach hohen Dosen liess sich eine schwache analgetische Wirkung feststellen. Nach parenteralen Dosen trat neuromuskuläre Lähmung ein. Letale Dosen führten den Tod durch periphere Atemlähmung oder Herzinsuffizienz herbei. Einige der Verbindungen besassen schwache cytostatische Eigenschaften. Die Verbindungen zeigten keine Schutzwirkung gegen letale Strahlungsdosen.

N,N,N',N'-Tetramethylthioharnstoff,[7] *N,N,N',N'*-Bis-(pentamethylen)-thioharnstoff [8] und *N,N',N',N*-Bis-(3-oxapentamethylen)-thioharnstoff [9] wurden nach bekannten Methoden durch Pyrolyse der entsprechenden Thiuramdisulfide dargestellt. *N,N,N',N'*-Bis-(3-oxapentamethylen)-thioharnstoff, der in der Literatur nur als Hydrat beschrieben war,[9] wurde durch Trocknen des Hydrats im Vakuumexsikkator, zuerst über konzentrierter Schwefelsäure, dann über Phosphorpentoxid, erhalten. F: 88,5–90°. (Gef. C 50,62; H 7,59. Ber. für $C_9H_{16}N_2O_2S$: C 49,97; H 7,46).

Die Thiouroniumsalze wurden im Falle $n = 0$ durch Umsetzung des entsprechenden Thioharnstoffs in Tetrachlorkohlenstoff mit der berechneten Menge Brom, in den anderen Fällen durch Erwärmen des entsprechenden Thioharnstoffs mit dem betreffenden Halogenid dargestellt und durch Umfällen aus absolutem Alkohol und absolutem Äther gereinigt.

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Oxidative Phosphorylation Accompanying the β-Oxidation of Carnitine-bound Fatty Acids

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It has recently been demonstrated that acylcarnitines are oxidized by liver mitochondria showing good respiratory control.[1] However, to our knowledge no experimental demonstration of the efficiency of oxidative phosphorylation during the isolated β-oxidation has appeared. The theoretical P:O ratio of the isolated β-oxidation of fatty acids is generally held to be 2.5, since the P:O ratio is 3 for the NAD-linked step, and, most likely, 2 for the flavoprotein step.[2] The present report deals with the phosphorylation coupled to the oxidation of carnitine-bound fatty acids by rat liver mitochondria when the citric acid cycle is blocked by malonate. As the acylcarnitines represent activated fatty acids,[3] their oxidation does not depend upon the availability of ATP for their activation. The use of an efficient ATP-trapping system therefore does not affect the oxidation of carnitine-bound fatty acids.

Table 1. Oxidative phosphorylation during β -oxidation.

Fresh rat liver mitochondria corresponding to 0.4 g of tissue (wet weight) were used. After 7–8 min of temperature equilibration, incubations were performed at 30° for 12 or 15 min. The main compartment contained: Mitochondria; potassium phosphate buffer, pH 7.1 (20 μmoles in expts. 1 and 3, 30 μmoles in expt. 2); $MgCl_2$ (15 μmoles); ATP (2.5 μmoles); albumin (30 mg); potassium chloride 0.15 M adjusting the final volume to 3 ml. The side arm contained: Hexokinase (1.5 mg) + glucose (100 μmoles); substrate; malonate (20 μmoles) when succinate was not the substrate; and Amytal, when added.

Expt. No.	Substrate (μmoles)	O_2-uptake (μatoms)	P_i esterified (μmoles)	P:O ratio
	Succinate [a] (60)	10.5	18.6	1.8
1	Pyruvate (30)	3.2	9.1	2.8
	Caprinylcarnitine (1.2)	7.0	13.4	1.9
	Palmitylcarnitine (0.6)	6.8	15.9	2.3
	Succinate (10)	12.2	22.3	1.8
2	Caprinylcarnitine (2)	7.9	15.2	1.9
	Palmitylcarnitine (1)	5.1	2.3	0.5
	Succinate [a] (60)	10.3	18.6	1.8
3	Succinate [a] + Amytal (2 mM) (60)	9.7	18.6	1.9
	Palmitylcarnitine (0.6)	5.3	11.1	2.1
	Palmitylcarnitine [b] (0.6) + Amytal (2 mM)	2.6	0	0

a Mitochondria corresponding to only 0.2 g of liver tissue were used.
b NAD (6 μmoles) was also added.

The preparation of carnitine esters was performed as earlier described.[3] Other reagents were commercial products of highest purity. Rat liver mitochondria were prepared as described by Myers and Slater.[4] The oxygen uptake was measured with conventional Warburg techniques. The reaction medium is stated in the text to the tables. The esterification of phosphate was calculated from the disappearance of P_i, determined by the method of Martin and Doty.[5]

Table 1 shows the oxidative phosphorylation with caprinylcarnitine or palmitylcarnitine as substrate in the presence of malonate, which blocks the oxidation of endogenous citric cycle intermediates in the mitochondria. In all experiments, the P:O ratio observed with acylcarnitines was higher than with succinate, but lower than with pyruvate. The highest P:O ratio observed during β-oxidation was 2.3, which was observed with palmitylcarnitine at a relatively low concentration (0.2 mM). Higher concentrations of palmitylcarnitine, which is a strong surface active agent, tended to uncouple oxidative phosphorylation (Table 1, expt. 2). At lower concentrations, this tendency could be counteracted by the addition of albumine to the medium. With caprinylcarnitine the highest P:O ratio observed was 1.9, and no uncoupling effect was seen at concentrations below 0.7 mM. Attempts were also made to

Table 2. Respiratory control during isolated β-oxidation.

The experimental conditions were as those given in Table 1. Malonate (20 μmoles) was added to all flasks. In vessels without acceptor system (expt. a) ATP, phosphate, hexokinase, and glucose were omitted. In expt. b, oligomycin (2 μg) was added to the main chamber, 2,4-dinitrophenol (10^{-4} M) from the side arm at the start of the experimental period.

Expt. a: Dependence of phosphate acceptor system:

Substrate (μmoles)	Phosphate and acceptor system	Oxygen uptake (μatoms)	Respiratory stimulation (%)	Acceptor control ratio	P:O ratio
Endogenous	−	0.4			
	+	1.2	200	3.0	2.5
DL-β-Hydroxybutyrate (20)	−	1.1			
	+	3.1	182	2.8	2.1
Caprinylcarnitine (1.2)	−	2.1			
	+	5.8	176	2.8	1.6

Expt. b: Inhibition of oxidation by oligomycin:

Substrate (μmoles)	Oligomycin	DNP	Oxygen uptake (μatoms)	P_i esterified (μmoles)	P:O ratio
Endogenous	−	−	1.7	0.7	0.4
	+	−	0.7	0	0
Palmitylcarnitine (0.6)	−	−	6.6	10.5	1.6
	+	−	2.5	0.5	0.2
	+	+	6.0	0	0

demonstrate the P:O ratio of the isolated flavoprotein step of β-oxidation. In these experiments, the oxidation of NADH was blocked by Amytal, and the mitochondria were supported with a NAD-regenerating system or external NAD in excess. The β-oxidation was thereby allowed to proceed through "the spiral". However, we did not succeed in demonstrating any significant phosphorylation by the mitochondria under these conditions, probably because palmitylcarnitine in the combination with Amytal causes uncoupling (Table 1, expt. 3). On the other hand, Amytal did not cause any reduction of the P:O ratio with succinate, probably because with these high amounts of succinate (60 μmoles), the oxidation of malate contributes very little to the P:O ratio measured also in the absence of Amytal.[6]

Table 2 a shows that caprinylcarnitine was oxidized by liver mitochondria with good respiratory control. The acceptor control ratio was equal to that observed when DL-β-hydroxybutyrate was the substrate. In accordance with this observation, Table 2 b shows an inhibition of the oxidation of palmitylcarnitine with oligomycin. This inhibition could be counteracted with 2,4-dinitrophenol.

The present study thus demonstrates that carnitine-bound fatty acids are oxidized by isolated mitochondria showing good respiratory control with a P:O ratio intermediary to that when succinate or pyruvate is the substrate. P:O ratios well above 2.0 were obtained. The theoretical P:O ratio of 2 for the isolated flavoprotein step of β-oxidation therefore gains support from our results.

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