Biochimica et Biophysica Acta, 357 (1974) 193–203

BBA 46784

KETOGENESIS IN ISOLATED RAT-LIVER MITOCHONDRIA

IV. OXALOACETATE DECARBOXYLATION: CONSEQUENCES FOR METABOLIC CALCULATIONS*

M. LOPES-CARDOZO and S. G. VAN DEN BERGH

Laboratory of Veterinary Biochemistry, State University of Utrecht, Biltstraat 172, Utrecht (The Netherlands)

(Received February 5th, 1974)

SUMMARY

Oxaloacetate which is formed by isolated rat-liver mitochondria during oxidation of malate may be decarboxylated to pyruvate by the action of oxaloacetate decarboxylase (EC 4.1.1.3). The pyruvate so formed is rapidly oxidized to acetyl-CoA from which citrate is formed by condensation with a second molecule of oxaloacetate. The rate of this pathway has been measured in various energy states in the presence of fluorocitrate to block the oxidation of citrate. The rate of conversion of malate to acetyl-CoA was calculated either from a comparison of the disappearance of malate and the formation of the various products or from $^{14}CO_2$ production from L-[U-^{14}C]-malate.

When fatty acids or pyruvate are oxidized together with malate, the conversion of malate to acetyl-CoA is decreased, especially in a high-energy state (State 4). In a low-energy state (State 3) this conversion decreases with increasing rates of β-oxidation; at the same time the concentration of oxaloacetate in the medium is lowered.

It is concluded tentatively that the rate of decarboxylation of oxaloacetate is controlled by the amount of oxaloacetate available to oxaloacetate decarboxylase in the mitochondrial matrix. Pyruvate was found to exert a direct inhibitory effect upon the decarboxylase. The implications of our observations for metabolic calculations concerning the simultaneous oxidation of fatty acid and malate are discussed. It is pointed out that relative rates of ketogenesis, calculated from oxygen uptake and disappearance of substrate, are severely overestimated if the variable contribution of malate to the acetyl-CoA pool is not taken into account.

INTRODUCTION

Isolated rat-liver mitochondria can oxidize added L-malate at an appreciable rate. Consequently, oxaloacetate is removed from the matrix compartment at a constant rate. Several pathways for this removal are known (Fig. 1). The formation of

* For papers I, II and III of this series see refs 10, 11 and 12.
Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

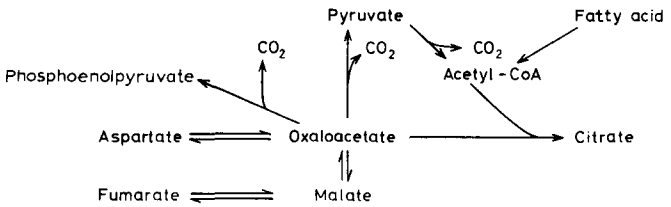

Fig. 1. Pathways for the removal of oxaloacetate in rat-liver mitochondria.

aspartate cannot proceed without added glutamate. The synthesis of phosphoenolpyruvate can contribute only very little to the removal of oxaloacetate due to the low activity of mitochondrial phosphoenolpyruvate carboxykinase (EC 4.1.1.32) in rat liver [1]. An efflux of oxaloacetate from the matrix compartment to the medium has been demonstrated recently [2]. However, during malate oxidation very little oxaloacetate accumulates in the medium.

Another pathway for removal of oxaloacetate could be its decarboxylation to pyruvate, catalyzed by oxaloacetate decarboxylase (EC 4.1.1.3), which is present in liver mitochondria of the rat [3–6].

When pyruvate oxidation is blocked by arsenite, CO_2 and pyruvate are formed in stoichiometric amounts from added oxaloacetate [4]. Oestreicher [7] has provided evidence that oxaloacetate decarboxylase is partly responsible for the removal of intramitochondrially generated oxaloacetate during oxidation of succinate or malate. Furthermore, Gimpel [2] has demonstrated that the removal of oxaloacetate via decarboxylation is probably responsible for the spontaneous reactivation of succinate oxidation in the presence of an uncoupler, rotenone and oxaloacetate [8].

This paper is concerned with the control of oxaloacetate decarboxylation during malate oxidation, especially when fatty acids or pyruvate are oxidized simultaneously. It will be shown that the decarboxylation of oxaloacetate is lowered during the oxidation of pyruvate or fatty acids, especially in a high-energy state (State 4). It is postulated that the rate of oxaloacetate decarboxylation is controlled by the amount of oxaloacetate available to the decarboxylase in the matrix compartment of the mitochondrion.

A preliminary report of this work has been published in abstract form [9].

METHODS

Incubations were carried out at 25 °C and the reactions were started by the addition of the mitochondria. The rate of conversion of malate to acetyl-CoA was calculated from the disappearance of malate and the formation of fumarate, phosphoenolpyruvate and citrate. At the end of the incubations 0.4 M $HClO_4$ (final concentration) was added and metabolites were measured spectrophotometrically, using standard enzymic methods [10].

Alternatively, $^{14}CO_2$ production from L-[U-^{14}C]malate was taken as an index of oxaloacetate decarboxylation. $^{14}CO_2$ was collected in 0.1 ml 10 M KOH during 2 h after the addition of $HClO_4$. The KOH was transferred to a counting vial with 5 ml water and 10 ml Instagel (Packard) was added. The radioactivity was measured

after an equilibration period of 12 h in a liquid scintillation spectrometer (Packard, Tri-Carb 2425) using an external standard for quench correction.

In all experiments 0.1 mM potassium fluorocitrate was added to prevent the oxidation of citrate.

Radiochemicals were obtained from Amersham Radiochemical Center. Other chemicals and experimental details were as previously described [10, 11].

RESULTS

Products of malate oxidation in various energy states

When rat-liver mitochondria are incubated in the presence of malate and fluorocitrate, only fumarate, phosphoenolpyruvate and citrate accumulate. These products have been measured in the experiment described in Table I. In this experiment the sum of malate plus fumarate was assayed but in other experiments (cf. Fig. 2) it was found that after about 10 min incubation fumarate is a constant fraction (20–25 %) of the malate in the medium. Pyruvate, oxaloacetate and acetyl-CoA do not accumulate to any extent (<0.1 nmole/min per mg protein).

TABLE I

MALATE OXIDATION IN VARIOUS ENERGY STATES

Mitochondria (16.5 mg protein) were incubated during 30 min in Warburg vessels in 1 ml of the standard reaction medium containing 50 mM sucrose, 5 mM $MgCl_2$, 2 mM EDTA, 15 mM KCl, 50 mM Tris–HCl, 0.5 mM ATP, 40 mM glucose, 40 mM potassium phosphate buffer (pH 7.5) and 0.1 mM potassium fluorocitrate. 2 units hexokinase (EC 2.7.1.1), CCCP (1 μM), oligomycin (10 μg) and ATP (10 mM) were added as indicated. After a preincubation of 2.5 min 10 mM L-malate was added from the sidearm. The production of citrate ($\triangle$ Cit), phosphoenolpyruvate ($\triangle$ PEP), fumarate ($\triangle$ Fum) and the disappearance of malate ($-\triangle$ Mal) were measured enzymically. Oxygen uptake was measured in a Gilson respirometer. Column D, $-\triangle$ (Mal–Fum–PEP) represents the disappearance of malate, corrected for $\triangle$ Fum and $\triangle$ PEP. Column E (Mal → AcCoA) gives the contribution of malate to the acetyl-CoA pool, calculated as the difference of citrate production and malate disappearance, corrected for $\triangle$ Fum and $\triangle$ PEP, (D–B). Under F the calculated contribution of endogenous substrates to the acetyl-CoA pool is listed ($AcCoA_{ES}$ = B–E). Column G represents the calculated oxygen uptake, $[\triangle O_2]_c = (C+3E+2.75F)/2$. Results are expressed as nmoles/min per mg mitochondrial protein.

Additions	A $-\triangle O_2$	B $\triangle$ Cit	C $\triangle$ PEP	D $-\triangle$ (Mal–Fum–PEP)	E Mal → AcCoA	F $AcCoA_{ES}$	G $[\triangle O_2]_c$
–	6.2	4.12	0.57	7.38	3.27	0.85	6.4
Hexokinase	11.9	8.32	0.18	14.23	5.91	2.41	12.3
CCCP	9.4	6.47	0.07	12.29	5.82	0.65	9.6
CCCP+oligomycin	10.3	6.66	0.04	12.18	5.52	1.14	9.9
CCCP+oligomycin +ATP	10.6	6.42	0.86	11.87	5.45	0.97	9.7

Column D of Table I shows the malate disappearance corrected for the production of fumarate and phosphoenolpyruvate. A comparison of Column D with the rate of citrate production (Column B) shows that more malate has disappeared than is accounted for by the production of fumarate, phosphoenolpyruvate and citrate.

This indicates that malate is converted to pyruvate, which in turn is decarboxylated to acetyl-CoA. In the presence of excess malate and a low NADH/NAD^+ ratio, acetyl-CoA is rapidly incorporated into citrate [10]. The rate of acetyl-CoA production from malate is assessed in Table I from the difference between the rate of malate disappearance and the rate of production of fumarate, phosphoenolpyruvate and citrate (Column E).

Table I also shows that more citrate is produced than the calculated conversion of malate to acetyl-CoA. Apparently, acetyl-CoA is mobilized from an endogenous source (Column F), presumably fatty acids [8].

The effect of palmitoylcarnitine on the products of malate oxidation

Fig. 2 shows the time course of malate oxidation in a high-energy state (State 4) and the effect of palmitoylcarnitine. In the absence of palmitoylcarnitine (Fig. 2A) phosphoenolpyruvate is produced (cf. Table I) showing that oxaloacetate is available to phosphoenolpyruvate carboxykinase. Ketone bodies do not accumulate. Apparently, the acetyl-CoA derived from malate is used completely for the synthesis of citrate and an overflow [12] towards the synthesis of ketone bodies does not occur.

After 10 min, malate is in equilibrium with fumarate via the reaction catalyzed by fumarate hydratase (EC 4.2.1.2). The ratio of malate disappearance corrected for the formation of oxaloacetate, pyruvate, phosphoenolpyruvate and fumarate (M_c, dotted line) over citrate production (Cit) is diagnostic for the source of acetyl-CoA used in citrate production. A ratio of 2 indicates that the acetyl-CoA is derived entirely

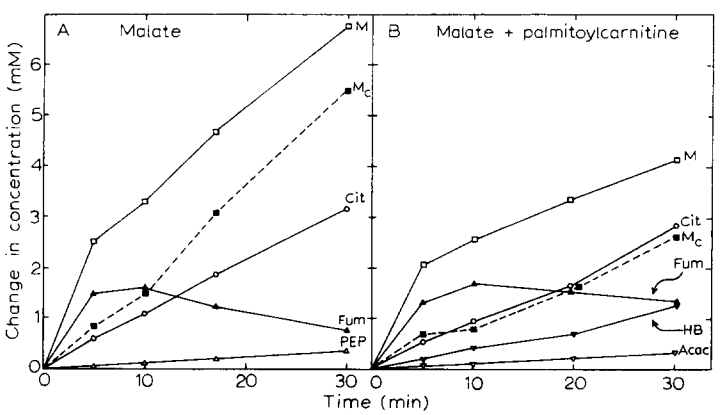

Fig. 2. Effect of palmitoylcarnitine on the State-4 oxidation of malate. Mitochondria, 10 mg (A) or 7.5 mg (B) protein/ml, were incubated in the standard reaction medium (Table I). After a preincubation period of 2.5 min, 10 mM L-malate was added. In Experiment B 0.5 mM L-palmitoylcarnitine and 0.8% (w/v) albumin were added with the malate. 1-ml samples were withdrawn at the times indicated. The disappearance of malate (M) (□—□), and the production of citrate (Cit) (○—○), acetoacetate (Acac) (▽—▽), 3-hydroxybutyrate (HB) (▼—▼), and fumarate (Fum) (▲—▲) were measured on a Zeiss PMQ II spectrophotometer. The production of pyruvate, phosphoenolpyruvate (PEP) (△—△), and oxaloacetate was measured in a combined assay with an Aminco–Chance dual-wavelength spectrophotometer. M_c (■...■) represents the disappearance of malate corrected for the production of fumarate, phosphoenolpyruvate (PEP), oxaloacetate and pyruvate.

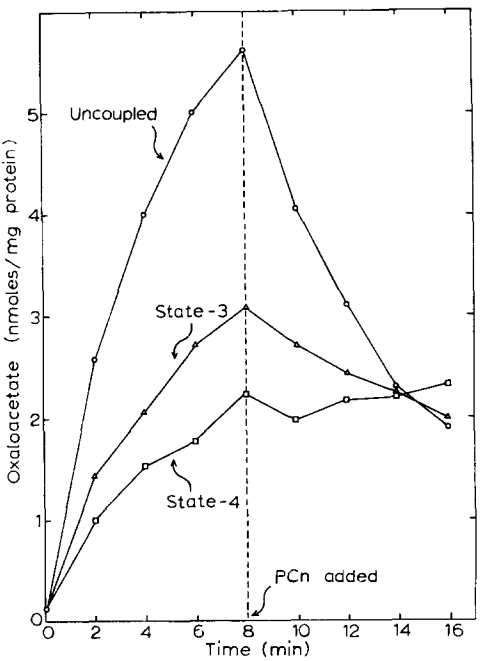

Fig. 3. Oxaloacetate levels during malate oxidation in various energy states, and the effect of palmitoylcarnitine. Mitochondria (5 mg protein/ml) were incubated in 125-ml Erlenmeyer flasks in a total volume of 20 ml of the standard reaction medium (Table I) from which fluorocitrate was omitted and to which 5 mM L-malate was added. Other additions: □—□, 0.5% (w/v) albumin; △—△, 0.5% (w/v) albumin and hexokinase (2.6 units/ml); ○—○, 10 μM pentachlorophenol (in this case potassium phosphate was omitted from the medium). After 8 min 0.12 mM L-palmitoylcarnitine (PCn) was added. At the times indicated 2-ml samples were withdrawn for the assay of oxaloacetate.

from malate via the formation of pyruvate. A ratio of 1 on the other hand shows that the production of acetyl-CoA out of malate is completely suppressed. In Fig. 2A the ratio M_c/Cit is initially 1.2. After about 10 min a ratio of 2 can be observed. This phenomenon can be explained by the presence of an endogenous source of acetyl-CoA [8] which is depleted during the first 10 min (cf. Table I). After 10 min, acetyl-CoA is generated only from added malate.

In Fig. 2B, in the presence of palmitoylcarnitine, the ratio M_c/Cit is close to 1 over the whole incubation period, suggesting that malate does not contribute to the acetyl-CoA pool during the oxidation of palmitoylcarnitine. Accumulation of pyruvate was not observed. It can be concluded, therefore, that the decarboxylation of oxaloacetate is inhibited during the State-4 oxidation of palmitoylcarnitine. It is worthwhile to note that the production of phosphoenolpyruvate was also completely inhibited in the presence of palmitoylcarnitine. Ketone bodies did accumulate, however. These results suggest that the activity of phosphoenolpyruvate carboxykinase and citrate synthase (EC 4.1.3.7) are limited by the availability of oxaloacetate. The oxaloacetate level at the end of the incubation period was 48 and 6 μM in the experiments shown in Figs 2A and 2B, respectively. Fig. 3 shows the accumulation of oxalo-

acetate during malate oxidation in various energy states. In the presence of an uncoupler more oxaloacetate is formed than during State-3 and State-4 oxidations of malate. Although the observed values are in line with the expected concentrations (see also Table II), they do not necessarily represent the oxaloacetate level in the matrix compartment of the mitochondrion. In agreement with previous reports [2, 7], we found that virtually all the oxaloacetate is in the extramitochondrial space: addition of NADH plus malate dehydrogenase resulted in an immediate drop of the oxaloacetate concentration to an undetectable level (<0.1 μM). When palmitoylcarnitine is added the oxaloacetate level is rapidly lowered in the case of uncoupled mitochondria and to a minor extent with coupled mitochondria in the presence of hexokinase (State 3).

$^{14}CO_2$ production during oxidation of L-[*U*-^{14}*C*]*malate*

A direct method to estimate the decarboxylation reactions depicted in Fig. 1 is the measurement of $^{14}CO_2$ production during oxidation of L-[U-^{14}C]malate. Table II shows the effect of palmitate oxidation upon the production of $^{14}CO_2$. The rate of palmitate oxidation was gradually enhanced by increasing the L-carnitine

TABLE II

THE RELATIONSHIP BETWEEN THE RATE OF PALMITATE OXIDATION AND THE CONVERSION OF MALATE TO ACETYL-CoA

Mitochondria (2.44 mg protein) were incubated during 20 min in 1 ml of the standard reaction medium (Table I) supplemented with 5 mM L-[U-^{14}C]malate (0.02 Ci/mole) and hexokinase (1.3 units). 1 mM arsenite, 0.8 mM [9,10-$^{3}H_2$]palmitate (1 Ci/mole) complexed to albumin (2%, w/v, final concentration), 50 μM CoASH and L-carnitine were added as indicated in the Table. The rate of palmitate oxidation was calculated from the water-soluble ^{3}H counts. Control experiments showed that in the presence of 1% (w/v) albumin more than 99% of the added palmitate is recovered in the pellet after precipitation of protein with 0.4 M $HClO_4$. Equal oxidation rates were found with [9,10-$^{3}H_2$]palmitate and [1-^{14}C]palmitate as substrates, showing that the ^{3}H label does not affect the oxidation rate. Results are expressed as nmoles/min per mg mitochondrial protein.

Additions	Palmitate oxidized	Malate converted to acetyl-CoA	Pyruvate	Phosphoenol-pyruvate	Oxalo-acetate
–	–	7.54	0.04	0.61	0.50
Arsenite	–	3.85	3.34	0.08	0.86
Palmitate+CoASH+ 20 μM L-carnitine	1.11	6.09	0.09	0.76	0.28
Palmitate+CoASH+ 50 μM L-carnitine	1.69	5.35	0.06	0.76	0.18
Palmitate+CoASH+ 100 μM L-carnitine	1.89	4.59	0.04	0.66	0.12
Palmitate+CoASH+ 1000 μM L-carnitine	2.68	3.44	0.06	0.64	0.11
Palmitate+CoASH+ 1000 μM L-carnitine*	3.55	2.11	0.03	0.16	0.07

* Final concentration of albumin was 1% (w/v).

concentration and by lowering the ratio of albumin over palmitate [11]. An increase of the carnitine concentration from 20 to 1000 μM stimulated palmitate oxidation more than 2-fold and a concurrent inhibition of the conversion of malate to acetyl-CoA can be observed. Lowering the albumin concentration (last line) results in an additional increase in the rate of palmitate oxidation and a further impairment of $^{14}CO_2$ production. CO_2 is also produced when oxaloacetate is converted to phosphoenolpyruvate. Table II shows, however, that the formation of phosphoenolpyruvate is only very small as compared to the conversion of malate to acetyl-CoA and accounts maximally for about 10 % of the removal of oxaloacetate in State 3 (cf. Fig. 2).

TABLE III

THE EFFECTS OF PALMITOYLCARNITINE AND OCTANOATE ON THE CONVERSION OF MALATE TO ACETYL-CoA IN VARIOUS ENERGY STATES

Mitochondria (7.32 mg protein) were incubated in 1.9 ml of the standard reaction medium (Table I) supplemented with 3 mM L-malate and 0.5% (w/v) albumin. Other additions: hexokinase (2.6 units/ml), CCCP (20 μM), oligomycin (5 μg/ml) and ATP (4 mM). After 5 min 0.32 μCi L-[U-^{14}C]malate (35 Ci/mole) and, if indicated, 0.33 mM L-palmitoylcarnitine and 1 mM octanoate were added in a volume of 0.1 ml. 16 min after addition of the mitochondria the reactions were terminated by addition of $HClO_4$ and L-malate plus fumarate was assayed in the neutralized supernatant. The values in the Table have been corrected for the disappearance of unlabelled malate in the 5-min preincubation period. The conversion of malate to acetyl-CoA is calculated from the $^{14}CO_2$ production.

Additions	Disappearance of malate (nmoles/min per mg protein)	Conversion of malate to acetyl-CoA (nmoles/min per mg protein)	Inhibition (%)
–	5.02	2.47	–
L-palmitoylcarnitine	–	0.31	87
Octanoate	–	0.17	93
Hexokinase	14.48	6.05	–
Hexokinase + L-palmitoylcarnitine	–	1.09	82
Hexokinase + octanoate	–	1.73	71
CCCP	10.35	4.84	–
CCCP + L-palmitoylcarnitine	–	1.85	62
CCCP + oligomycin + ATP	7.95	3.73	–
CCCP + oligomycin + ATP + L-palmitoylcarnitine	–	1.59	57
CCCP + oligomycin + ATP + octanoate	–	1.66	55

In Table III it is shown that palmitoylcarnitine and octanoate are equally effective in suppressing CO_2 production from malate. Table III also shows that this inhibition is more pronounced in State 4 than in State3. Addition of the uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP) decreases the inhibition still further, whereas oligomycin and ATP have no significant effect on the percentage inhibition.

Pyruvate also inhibits $^{14}CO_2$ production from labelled malate (Table IV). As in the case of fatty acids the inhibition is most pronounced in a high-energy state

TABLE IV

THE EFFECTS OF PYRUVATE AND ARSENITE ON THE CONVERSION OF MALATE TO ACETYL-CoA

Mitochondria (4.12 mg protein) were incubated during 20 min in 1 ml of the standard reaction medium (Table I) supplemented with 5 mM L-[U-^{14}C]malate (0.02 Ci/mole). Hexokinase (2.6 units), 1 μM CCCP, 1 mM arsenite and 5 mM sodium pyruvate were added as indicated. The conversion of malate to acetyl-CoA was calculated from the $^{14}CO_2$ production

Additions	Conversion of malate to acetyl-CoA (nmoles/min per mg)	Inhibition (%)
–	2.38	–
Pyruvate	0.19	92
Hexokinase	5.47	–
Hexokinase + pyruvate	1.28	77
Hexokinase + arsenite	2.79	49
Hexokinase + arsenite + pyruvate	1.57	71
CCCP	4.74	–
CCCP + pyruvate	1.02	78

(State 4). Addition of arsenite suppresses the CO_2 production in State 3, presumably by inhibiting the oxidative decarboxylation of pyruvate (cf. Table II). Table IV further shows that, even in the presence of arsenite, pyruvate exerts an inhibitory effect on the CO_2 production during State-3 oxidation of malate. This observation points to a product inhibition of oxaloacetate decarboxylase by pyruvate.

Table V shows that pyruvate indeed inhibits the decarboxylase, assayed in sonicated mitochondria, with a K_i of about 4 mM (contrast ref. 6). Arsenite has no effect either on the rate of decarboxylation or on the degree of inhibition by pyruvate. The decarboxylase is independent of divalent cations (refs 5 and 6; contrast ref. 3) and is easily solubilized by sonication (results not shown).

TABLE V

INHIBITION OF OXALOACETATE DECARBOXYLASE BY PYRUVATE

Rat-liver mitochondria (200 mg protein) were suspended in 10 ml Tris–HCl (100 mM), EDTA (1 mM) and 1,4-dithioerythritol (1 mM), pH 7.4 and treated during four 1-min periods in a MSE sonic disintegrator. Oxaloacetate decarboxylase activity was assayed in the same medium in which oxaloacetate (2 mM) was included. After 20 min, $HClO_4$ (0.4 M final concentration) was added and oxaloacetate was assayed in the neutralized supernatant. The values in the Table have been corrected for non-enzymic breakdown of oxaloacetate.

Additions	Oxaloacetate decarboxylase activity (nmoles/min per mg mitochondrial protein)	
	Without arsenite	Plus arsenite (2.5 mM)
–	17.6	16.5
Pyruvate (1 mM)	13.5	13.1
Pyruvate (5 mM)	8.0	6.7

DISCUSSION

Control of oxaloacetate decarboxylation

During oxidation of malate by rat-liver mitochondria oxaloacetate is decarboxylated to pyruvate, which in turn contributes an acetyl group for the synthesis of citrate as depicted in Fig. 1. Arguments in favour of this pathway may be briefly summarized: (a) More malate disappears than can be accounted for by production of fumarate, phosphoenolpyruvate, and citrate (Table I). After some time the ratio of malate disappearance (corrected for fumarate and phosphoenolpyruvate production) over citrate formation is 2, suggesting that two molecules of malate are necessary for the synthesis of one molecule of citrate (Fig. 2). (b) During oxidation of L-[U-^{14}C]-malate in the presence of fluorocitrate, $^{14}CO_2$ is produced at a rate which exceeds at least 9-fold the rate of phosphoenolpyruvate formation (Table II). (c) In the presence of arsenite $^{14}CO_2$ production is lowered and pyruvate accumulates (Table II). These data confirm the work of Oestreicher [4] who observed that equal amounts of pyruvate and CO_2 were generated from oxaloacetate in the presence of arsenite. (d) An oxaloacetate decarboxylase has been demonstrated in rat-liver mitochondria [3–6].

We have shown that the decarboxylation of oxaloacetate is reduced during oxidation of palmitoylcarnitine (Fig. 2, Table III), octanoate (Table III), palmitate (Table II) and pyruvate (Table IV), the degree of reduction being dependent on the mitochondrial energy state (Table III: State 4 > State 3 > uncoupled state). Under State-3 conditions the rate of the decarboxylation reactions is inversely related to the rate of palmitate oxidation; this effect is not brought about by a palmitate-induced inhibition of pyruvate oxidation [13], since no pyruvate accumulation is found (Table II).

These results point to a possible control of oxaloacetate decarboxylation by the availability of oxaloacetate to the decarboxylase. This hypothesis is supported by the observation that the oxaloacetate concentration in the medium is lowered upon addition of palmitoylcarnitine to mitochondria oxidizing malate in a low-energy state (Fig. 3). The observed level of oxaloacetate is correlated with the rate of decarboxylation and is inversely related to the rate of β-oxidation (Table II). These findings suggest that, at least in a low-energy state, oxaloacetate in the medium is in equilibrium with a very small pool of oxaloacetate in the mitochondrial matrix.

In a high-energy state (State 4) fatty acids reduce oxaloacetate decarboxylation to a very low rate (Table III, Fig. 2) by inducing a high NADH/NAD$^+$ ratio and thereby presumably lowering the oxaloacetate level in the matrix.

Pyruvate strongly lowers $^{14}CO_2$ production from L-[U-^{14}C]malate (Table IV) even in the presence of arsenite, indicating that pyruvate directly inhibits the decarboxylase. This hypothesis is supported by the observed inhibition of oxaloacetate decarboxylation by pyruvate in sonicated mitochondria (Table V; contrast ref. 6).

In short, we conclude that in the absence of fatty acids and pyruvate, oxaloacetate is converted to pyruvate, which in turn provides acetyl-CoA for the synthesis of citrate. When fatty acids or pyruvate are added, the rate through this pathway decreases presumably by a decreased amount of oxaloacetate available to the decarboxylase. The data presented in Tables IV and V suggest that pyruvate exerts a feedback control on the decarboxylation of oxaloacetate.

Implications for metabolic calculations

The conclusion that the production of acetyl-CoA from Krebs cycle intermediates via two subsequent decarboxylations of oxaloacetate and pyruvate, respectively, is diminished by fatty acids and by pyruvate, has some practical implications which are illustrated in Fig. 4.

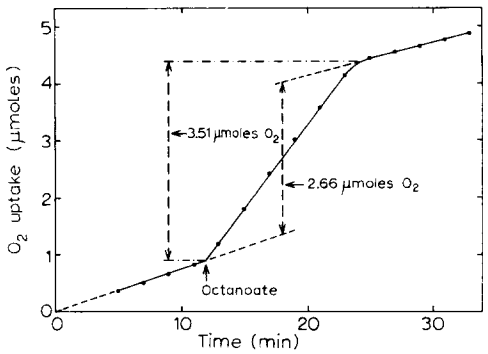

Fig. 4. Two methods for the calculation of the oxygen consumed in the oxidation of a limited amount of octanoate in the presence of L-malate. Mitochondria (7.14 mg protein) were incubated in 1.0 m of the standard reaction medium (Table I) supplemented with hexokinase (2.6 units) and 5 mM L-malate. After 12 min, 0.75 μmole octanoate was added. After 34 min the reaction was stopped by addition of $HClO_4$ and acetoacetate (0.37 μmole) and 3-hydroxybutyrate (< 0.01 μmole) were assayed in the neutralized supernatants. Oxygen uptake was measured with differential manometers with narrow capillaries and gas volumes of 4–5 ml. The oxygen consumption for the oxidation of the added octanoate can be calculated as follows: During octanoate oxidation 0.37 μmole acetoacetate was formed (3-hydroxybutyrate does not accumulate under State-3 conditions). The added octanoate is equivalent to 3 μmoles of acetyl groups. Therefore $3-(2\times0.37) = 2.26$ μmoles citrate must be produced during octanoate oxidation. The calculated oxygen uptake is then $1.5\times0.37+1.25\times2.26 = 3.38$ μmoles.

In this experiment mitochondria are incubated in the presence of malate, fluorocitrate and hexokinase (State 3). At $t = 12$ min a small amount of octanoate is added which is completely oxidized within 12 min, as is shown by the deflection in the oxygen uptake at $t = 24$ min. The oxygen uptake between $t = 0$ min and $t = 12$ min and between $t = 24$ min and $t = 33$ min is caused by a slow conversion of malate to citrate (Fig. 1). Ketone bodies are not formed during these periods due to the low rate of acetyl-CoA formation in a low-energy state [12] and the presence of excess malate [10]. During the period of octanoate oxidation acetyl-CoA is produced rapidly and is distributed between citrate and acetoacetate.

The amount of oxygen taken up during oxidation of a limited amount of fatty acid or pyruvate can be used to calculate this distribution. The group of Garland [13] has used this calculation extensively in their studies on the control of citrate and ketone body formation during oxidation of palmitoylcarnitine. Fig. 4 shows, however, that this oxygen uptake cannot be measured unambiguously in the presence of L-malate. Extrapolation of the rate of malate oxidation during the period of fatty acid oxidation implicitly assumes that the contribution of malate to the acetyl-CoA pool is not inhibited during octanoate oxidation. This assumption leads to an oxygen uptake of 2.66 μmoles. However, if we ignore a possible contribution of malate to the

acetyl-CoA pool during fatty acid oxidation we find another extreme value of 3.51 μmoles of oxygen taken up. The actual oxygen consumption for octanoate oxidation can be calculated from the oxidation products and amounts to 3.38 μmoles (see legend to Fig. 4).

It must be concluded that extrapolation of the oxygen uptake during malate oxidation into the period of oxidation of fatty acids plus malate, leads to an underestimation of the oxygen uptake for the oxidation of fatty acids [13, 14] and consequently to an overestimation of the relative rate of ketogenesis [13]. The distribution of acetyl-CoA over ketone bodies and citrate can be estimated accurately only by measuring the products of fatty acid oxidation [10].

NOTE ADDED IN PROOF

Recently, Wojtczak and Wałajtys [15] have reported a partial purification and characterization of oxaloacetate decarboxylase from rat liver mitochondria. The authors provide evidence that this activity is responsible for the removal of oxaloacetate during malate oxidation.

ACKNOWLEDGEMENTS

The excellent technical assistance of Miss W. Klazinga is gratefully acknowledged.

This investigation was supported in part by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

REFERENCES

1 Nordlie, R. C. and Lardy, H. A. (1963) J. Biol. Chem. 238, 2259–2263
2 Gimpel, J. A. (1973) Mitochondrial Processes Involving Oxaloacetate, Ph.D. Thesis, Amsterdam, Gerja, Waarland
3 Corwin, L. M. (1959) J. Biol. Chem. 234, 1338–1341
4 Oestreicher, A. B. (1970) Het Mechanisme van de Remming van de Succinaatoxidatie door Ontkoppelaars, Ph.D. Thesis, Amsterdam, Mondeel, Amsterdam
5 Wojtczak, A. B. and Wałajtys, E. (1973) Abstr. 9th Int. Congr. Biochem. Stockholm, p. 350
6 Dean, B. and Bartley, W. (1973) Biochem. J. 135, 667–672
7 Oestreicher, A. B., Van den Bergh, S. G. and Slater, E. C. (1969) Biochim. Biophys. Acta 180, 45–55
8 Wojtczak, A. B. (1969) Biochim. Biophys. Acta 172, 52–65
9 Lopes-Cardozo, M. and Van den Bergh, S. G. (1971) Abstr. Commun. 7th Meet. Eur. Biochem. Soc., p. 227
10 Lopes-Cardozo, M. and Van den Bergh, S. G. (1972) Biochim. Biophys. Acta 283, 1–15
11 Lopes-Cardozo, M. and Van den Bergh, S. G. (1974) Biochim. Biophys. Acta, 357, 43–52
12 Lopes-Cardozo, M. and Van den Bergh, S. G. (1974) Biochim. Biophys. Acta, 357, 53–62
13 Garland, P. B., Shepherd, D., Nicholls, D. G., Yates, D. W. and Ann Light, P. (1969) Citric Acid Cycle (Lowenstein, J. M., ed.), pp. 163–212, Marcel Dekker, New York
14 Bode, C. and Klingenberg, M. (1965) Biochem. Z. 341, 271–299
15 Wojtczak, A. B. and Wałajtys, E. (1974) Biochim. Biophys. Acta 347, 168–182