Diversity of Bacterial Respiratory Systems

Volume II

Editor

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PREFACE

Although wide differences occur in the composition and function of mitochondrial respiratory systems, there is also a distinct and fundamental similarity between them, whether they originate from plants, animals, or microorganisms. However, in bacteria, respiratory systems vary enormously in both composition and function from the very simple to complex mitochondrial-like systems, depending on the degree of evolutionary sophistication of the organism and the type of habitat in which they exist. For example, there are bacteria that respire with oxygen, nitrate, fumarate, or sulfur compounds as electron acceptors, whereas mitochondrial systems respire only to oxygen. Some bacteria even require reversal of electron transfer against the normal electrochemical gradient in order to grow.

It is the aim of this book to present reviews on a wide range of aspects of bacterial respiratory systems. Because of the on-going publication elsewhere of reviews on bacterial respiration, a "blanket" coverage of the field has not been attempted. Rather, a range of topics have been selected, either because they are of special current interest, they have not been reviewed recently, or they have never been reviewed.

C. J. Knowles

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Chapter 1

BACTERIAL ELECTRON TRANSPORT TO FUMARATE

A. Kröger

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I. INTRODUCTION

Until about 1970, it was generally believed that anaerobic nonphotosynthetic bacteria could synthesize ATP only by substrate level phosphorylation reactions and that the occurrence of electron transport phosphorylation was restricted to aerobic bacteria.[1] The reduction of nitrate as a known exception from this rule was thought to be a property of aerobic organisms only. In the meantime, it has been discovered that strictly anaerobic bacteria can derive the ATP required for growth from the reduction of fumarate, with either succinate or propionate as the end product. This was surprising in view of the redox potential of the fumarate/succinate couple which is nearly 400 mV more negative than that of nitrate/nitrite.

This chapter will concentrate mainly on two aspects:

1. The role of fumarate reduction in anaerobic metabolism
2. Fumarate reduction as a system of electron transport phosphorylation

Other aspects have been extensively discussed in recent reviews.[2-5]

II. THE ROLE OF FUMARATE IN ANAEROBIC METABOLISM

Hydrogen transfer is a general feature of aerobic and anaerobic metabolism. Substrate level phosphorylation, for instance, is often coupled to the oxidation of a carbon compound by the pyridine nucleotides. Under aerobic conditions, the reduced pyridine nucleotides are reoxidized by oxygen as an abundantly available acceptor with a sufficiently high redox potential (Table 1). An acceptor with similar properties is not at the disposal of anaerobic bacteria under most conditions. Although abundantly available from water dissociation, protons are rarely used as acceptors of reducing equivalents because of the low redox potential of the couple H^+/H_2 under the usually pH-neutral growth conditions. The production of molecular hydrogen from NADH and protons is an endergonic reaction which can occur only at the expense of other exergonic processes. CO_2 is expected to be available under most of the growth conditions of bacteria. However, the redox potential of bicarbonate with formate as the product of two-electron reduction is about as negative as that of protons. The redox potentials of the couples HCO^-_3/methane and HCO^-_3/acetate are distinctly more positive than those of H^+/H_2. Nevertheless, only methanobacteria and some other specialized organisms seem to be able to use CO_2 as a hydrogen acceptor and to derive useful energy from its reduction to methane or acetate.[2] In the fixation of CO_2 which is observed with many enteric bacteria fermenting sugars with succinate as one of the end products (see Figure 4B and C), CO_2 is not used as a true hydrogen acceptor. The fixed CO_2 is conserved in the carboxyl group of succinate and not further reduced. Sulfate and elemental sulfur can be used as hydrogen acceptors by certain specialized bacteria.[2] Sulfide is the end product in both cases. The redox potentials of sulfate and sulfur are in the same range as that of CO_2. In contrast to the acceptors mentioned above, nitrate exhibits an exceedingly high redox potential. Correspondingly a great variety of different bacteria have developed the ability to reduce nitrate either to nitrite or some other compounds.[2,4]

Most of the known anaerobic bacteria synthesize their hydrogen acceptors from the growth substrates. Pyruvate is a well-known example of a hydrogen acceptor which is reduced to lactate during the fermentation of glucose by many bacteria (see Table 1). Acrylate and crotonate appear to be more suitable hydrogen acceptors than pyruvate because their redox potentials are positive enough to oxidize most of the carbon com-

Table 1
REDOX COUPLES INVOLVED IN BACTERIAL ENERGY METABOLISM

	E'_0 (mV)
H^+/H_2	−420
HCO^-_3/formate	−416
NAD^+/NADH	−320
HCO^-_3/acetate	−280
S/HS^-	−274
HCO^-_3/CH_4	−244
$SO_4^{=}/HS^-$	−220
Pyruvate/lactate	−197
Dihydroxyacetone-P/glycerol-1-P	−190
Oxaloacetate/malate	−172
Acrylate/propionate	−30
Crotonate/butyrate	−30
Fumarate/succinate	+30
Nitrate/nitrite	+420
O_2/H_2O	+815

pounds involved in metabolism. The CoA-esters of acrylate and crotonate are well-known hydrogen acceptors in anaerobic bacteria that form fatty acids as end products.[2] The enzymes catalyzing the reduction reactions are soluble in contrast to fumarate reductase that is bound to the membrane.[2,6] Although the redox potential span between NADH and the CoA-esters of acrylate and crotonate is sufficient to allow the synthesis of 1 ATP/2e$^-$, electron transport phosphorylation has not been shown to be coupled to the reduction of these compounds.[2]

The redox potential of the couple fumarate/succinate is even more positive than that of acrylate/propionate (see Table 1). Therefore, fumarate is a suitable hydrogen acceptor in anaerobic metabolism. In the absence of acceptors with more positive redox potentials, succinate cannot further be metabolized and is therefore a true end product of fermentation. Fumarate reduction occurs more frequently in bacteria than fatty acid fermentation and is about as widespread as nitrate reduction.[4] The formation of propionate proceeds via fumarate and not with acrylate as the intermeditate in most of the bacteria. The reason for the widespread occurrence of fumarate reduction resides probably in its availability from a variety of organic compounds, like amino acids which are likely to be present in many biotopes, and in its ability of being coupled to electron transport phosphorylation. In the following, examples of metabolic pathways involving fumarate reduction are given.

While growth of *Escherichia coli*[7,8] and *Vibrio succinogenes*[9] with fumarate is sustained only in the presence of a hydrogen donor (see Table 2) *Proteus rettgeri*[10] (see Figure 1A) and *Clostridium formicoaceticum*[11] (see Figure 1B) can grow with fumarate as the only energy substrate. In both pathways, part of the fumarate is oxidized, and the hydrogen liberated in these branches of metabolism is used for the reduction of fumarate to succinate. With *C. formicoaceticum*, 1 mol acetate and 2 mol CO_2 are the end products of the oxidation of 1 mol fumarate, while 2 mol fumarate are oxidized to give 1 mol succinate and 4 mol CO_2 with *P. rettgeri*. In the latter case, the acetyl-CoA is used to form citrate which is then oxidized via part of the citric acid cycle. Malate is fermented in the same way as fumarate by *P. rettgeri*. In contrast, *C. formicoaceticum* produces mainly acetate and CO_2 from malate. In this pathway, part of the acetate is formed by CO_2 reduction.[11]

The pathway of fermentation of citrate by growing *P. rettgeri* (see Figure 2) is similar

to that of fumarate (see Figure 1A) in that part of the citrate is again oxidized via part of the citric acid cycle.[10] Fumarate is produced from oxaloacetate which is formed from citrate by the citrate lyase reaction. The formation of fumarate from oxaloacetate is a general process in those pathways by which glucose or C_3-compounds are fermented, with either succinate or propionate as end products (see Figures 3 and 4). These pathways may differ in the reactions by which oxaloacetate is formed. The pathways of fermentation of glycerol, lactate, and glucose[12,13] by growing propionic acid bacteria are given in Figures 3 and 4A. Propionic acid bacteria synthesize oxaloacetate from pyruvate by means of transcarboxylation.[12] In this reaction, methylmalonyl-CoA serves as the carboxyl donor to give propionyl-CoA. ATP is not required in this reaction. The succinate formed by fumarate reduction is subsequently converted to propionate.[12] The intermediates of this sequence are not given in the figures. In the first step of the sequence, succinyl-CoA is formed from succinate by transacylation with propionyl-CoA. This step does not require ATP. With glycerol as the growth substrate, the hydrogen required for the reduction of oxaloacetate is generated in the Emden-Meyerhof pathway alone, while with lactate and glucose, additional hydrogen is provided in the branch leading to acetate.

In the fermentation of glucose by growing *Bacteroides fragilis* (see Figure 4B), only part of the succinate is converted to propionate (not shown).[14,15] This means that the fermentation is associated with the fixation of CO_2. The CO_2 is incorporated into oxaloacetate which is synthesized from phosphoenolpyruvate with the concomitant phosphorylation of ADP. In further contrast to propionibacteria, the degradation of pyruvate to acetyl-CoA is not associated with NAD reduction, but yields formate as an end product instead.

Anaerobically growing enterobacteria form variable amounts of succinate from glucose.[16,17] As an example, the fermentation pathway of glucose in the presence of bicarbonate by *P. rettgeri*[10] is in Figure 4C. Oxaloacetate is probably synthesized from phosphoenolpyruvate without recovery of the phosphate bond. This is assumed because other enterobacteria were found to synthesize C_4 from C_3-compounds only via the phosphoenolpyruvate carboxylase reaction.[18-20] Part of the acetyl-CoA which is formed via pyruvate-formatelyase, is reduced to ethanol in *P. rettgeri*. In *E. coli*, malate is apparently not an obligatory intermediate of fumarate production, since a mutant that is deficient of malate dehydrogenase was found to synthesize fumarate from oxaloacetate via aspartate.[19]

III. GROWTH YIELDS

E. coli can grow in defined media at the expense of the reduction of fumarate by molecular hydrogen with succinate as the only product[7,8] (see Table 2). The same is true for *V. succinogenes*[9] and a strain of *Desulfovibrio*.[21] As the ATP required for growth cannot be derived from substrate level phosphorylation under these conditions, it is clear that the ATP must be formed by the electron transport phosphorylation coupled to fumarate reduction. A similar situation is valid for *V. succinogenes*, when growing on fumarate and formate with succinate and bicarbonate as the products (see Table 2). These cases demonstrate unambiguously that fumarate reduction can yield ATP via electron transport phosphorylation.

In the other cases in Table 2, the situation is complicated by the occurrence of substrate level phosphorylation reactions in addition to possible electron transport phosphorylation with fumarate. In these cases, the cell yields per mole of substrate (Y_s) have to be measured. Division of Y_s by the ATP yield per mole of substrate (n) which is expected from the corresponding metabolic pathway gives the cell yield per mole of

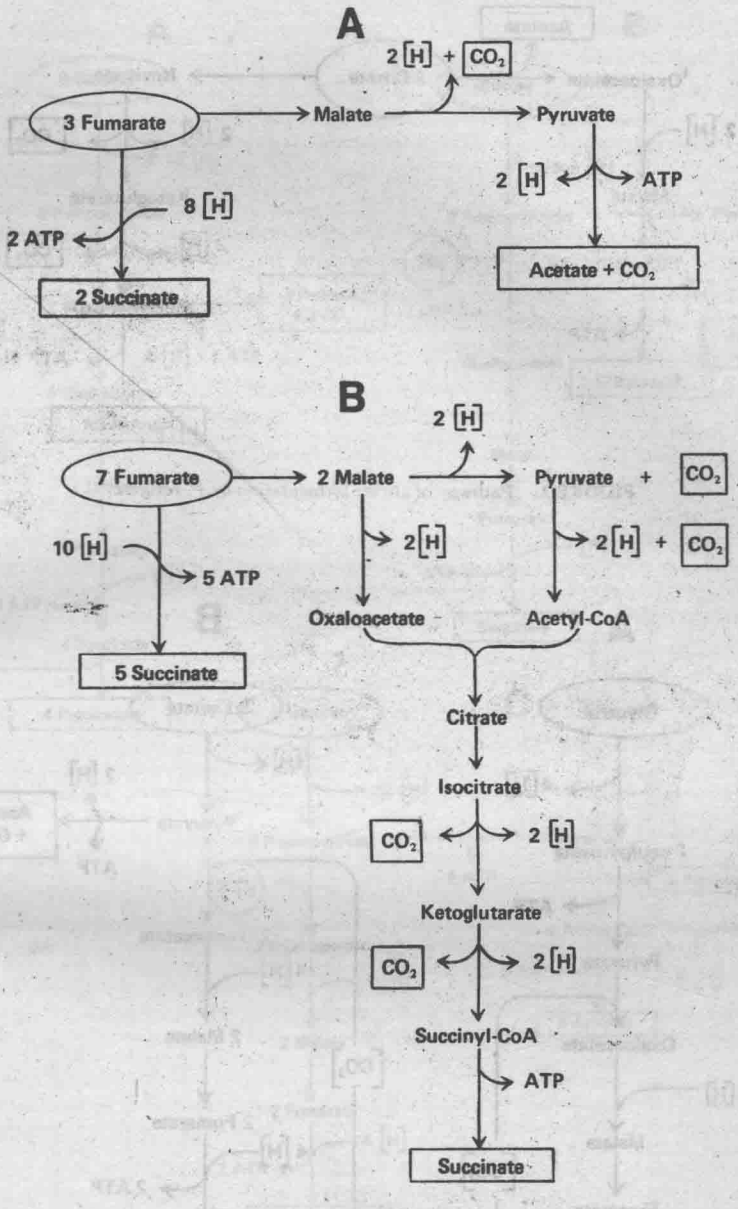

FIGURE 1. Pathway of fermentation of fumarate by (A) *C. formicoaceticum*[11] and (B) *P. rettgeri*.[10]

ATP (Y_s/n). As Y_s/n is believed to be a constant which is independent of the bacterial strain and the growth substrate under certain conditions[22-24] it is often possible to decide from growth yields whether fumarate reduction is associated with phosphorylation or not. The special implications of this method are discussed in detail by Neijssel and Tempest in Volume I, Chapter 1. The method is applied here to cases in which the growth reactions as well as the pathways of fermentation are known. The cell yields were obtained with batch cultures and the given numbers were not extrapolated for the "energy of maintenance".

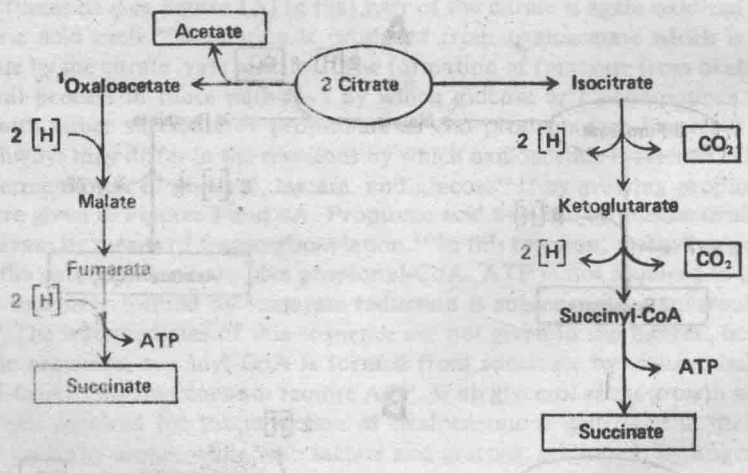

FIGURE 2. Pathway of citrate fermentation by *P. rettgeri*.[10]

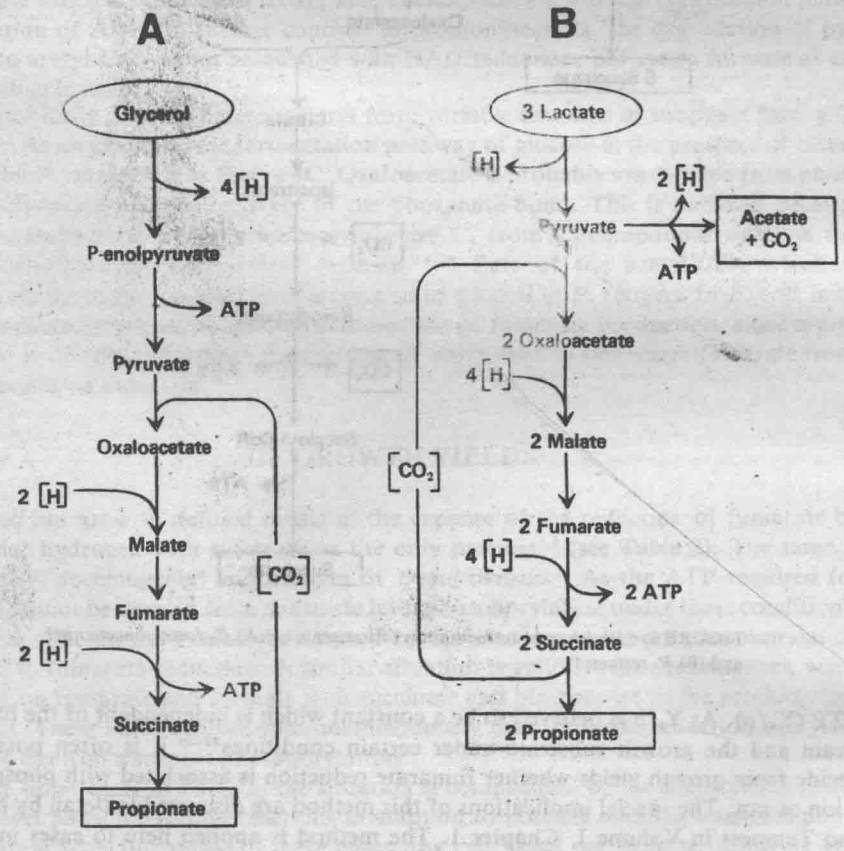

FIGURE 3. Fermentation pathways of (A) glycerol and (B) lactate of propionic acid bacteria.[12,13]

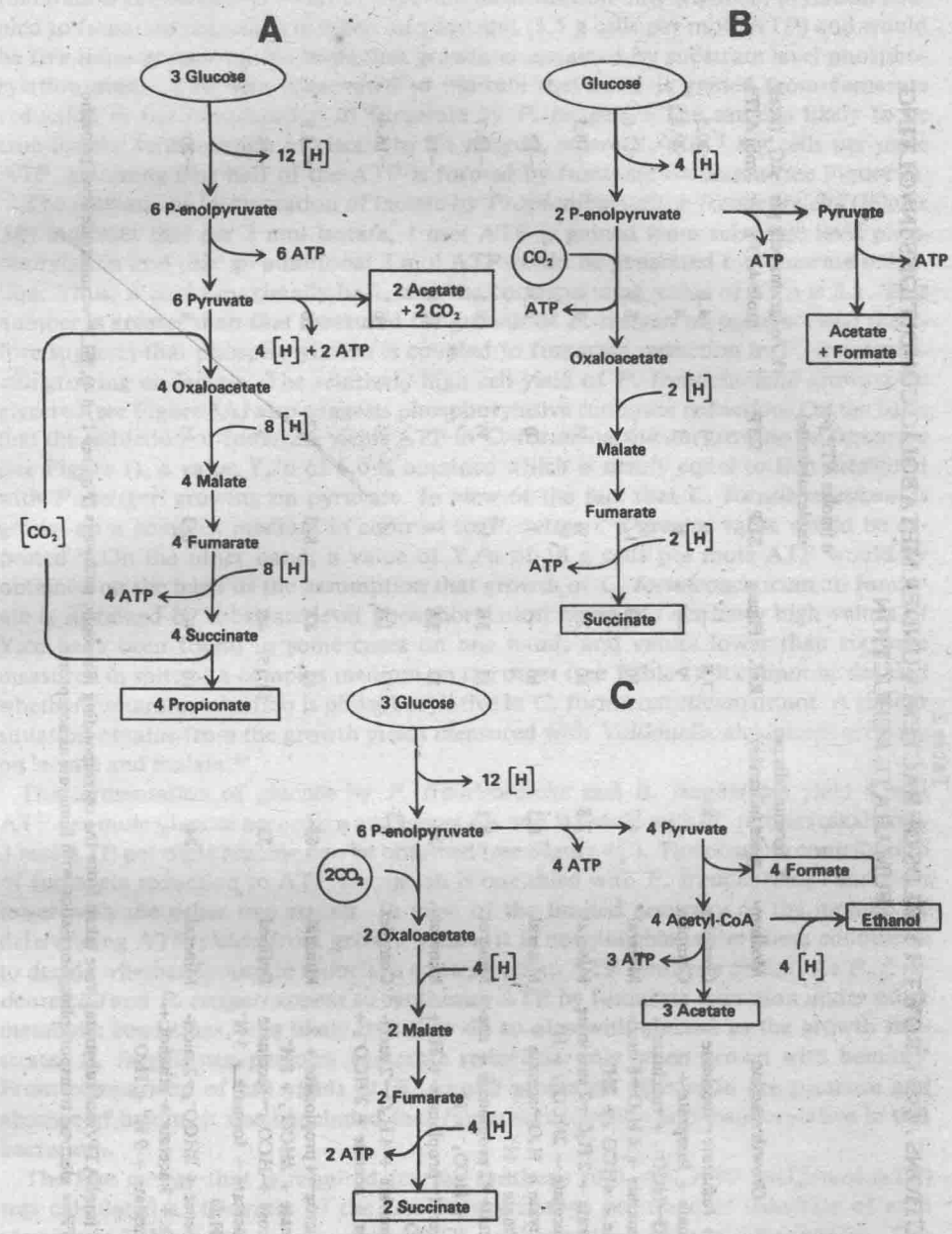

FIGURE 4. Pathways of glucose fermentation by (A) propionic acid bacteria,[12,13] (B) *B. fragilis*,[14,15] and (C) *P. rettgeri*.[10]

According to the pathway of Figure 1B, the fermentation of fumarate by *P. rettgeri* yields 1 and 5 mol ATP per 7 mol fumarate by substrate level and electron transport phosphorylation respectively. The cell yield obtained with pyruvate (6.3 g cells per mole pyruvate) is used for judging the ATP yield with fumarate as growth substrate. Pyruvate is fermented in a simple pathway to give formate and acetate, and it is evident that 1 mol ATP per mole pyruvate is formed. The cell yield per mole ATP (Y_{ATP}) with

Table 2

GROWTH REACTIONS AND GROWTH YIELDS OF BACTERIA UNDER METABOLIC CONDITIONS INVOLVING FUMARATE REDUCTION

Bacterial strain	Growth reaction	Medium	Y_s (grams cells per mole substrate)	Ref.	Figure	n (moles ATP per mole substrate) SLP	n (moles ATP per mole substrate) ETP	Y_s/n (grams cells per mole ATP)	$\Delta G'_s$ (kcal/mol ATP)
Escherichia coli	H_2 + fumarate→succinate	Complex	4.8	7,8	—	0	1	4.8	−20.5
Vibrio succinogenes	formate + fumarate + H_2O→HCO_3^- + succinate	Complex	4	9	—	0	1	4	−20.3
Proteus rettgeri	7 fumarate + 8 H_2O→6 succinate + 4HCO_3^- + 2H^+	Mineral	4.7	10	1B	1/7	5/7	5.5	−17.6
P. rettgeri	2 citrate + 2 H_2O→2 succinate + acetate + 2HCO_3^- + H^+	Mineral	7.8	10	2	½	½	7.8	−18.5
P. rettgeri	Pyruvate + H_2O→acetate + formate + H^+	Mineral	6.3	10	—	1	0	6.3	−12
P. freudenreichii	3 lactate→2 propionate + acetate + HCO_3^- + H^+	Mineral	8.1	13	3B	⅓	⅔	8.1	−13.7
P. freudenreichii	Glycerol→propionate + H^+ + H_2O	Mineral	24	13	3A	1	1	12	−18
Clostridium formicoaceticum	3 fumarate + 4 H_2O→2 succinate + acetate + 2 HCO_3^- + H^+	Complex	6	11	1A	⅓	⅔	6	−16.4
P. freudenreichii	3 glucose→4 propionate + 2 acetate + 2HCO_3^- + 8H^+	Mineral	65	13	4A	8/3	4/3	16	−18.8
Bacteroides fragilis	Glucose + HCO_3^-→succinate + acetate + formate + 3 H^+ + H_2O	Defined	53	14,15	4B	3	1	13	−16
P. rettgeri	3 glucose + 2HCO_3^-→2 succinate + 3 acetate + ethanol + 4 formate + 9 H^+ + H_2O	Mineral	24	10	4C	7/3	⅔	8	−20

Note: The values of n (mol ATP/mol substrate) were taken from the corresponding metabolic pathways (Figures 1 to 4). SLP = substrate level phosphorylation, and ETP = electron transport phosphorylation with fumarate as acceptor.