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Regulation of Energy Metabolism in Liver

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Energy metabolism in liver has to cope with the special tasks of this organ in intermediary metabolism. Main ATP-generating processes in the liver cell are the respiratory chain and glycolysis, whereas main ATP-consuming processes are gluconeogenesis, urea synthesis, protein synthesis, ATPases and mitochondrial proton leak. Mitochondrial respiratory chain in the intact liver cell is subject to control mainly by substrate (hydrogen donors, ADP, oxygen) transport and supply and proton leak/slip. Whereas hormonal control is mainly on substrate supply to mitochondria, proton leak/slip is supposed to play an important role in the modulation of the efficiency of oxidative phosphorylation.

KEY WORDS: Energy metabolism; liver mitochondria; metabolic control; nonequilibrium thermodynamics.

INTRODUCTION

The liver is a center of intermediary metabolism. It acts as the glucose reservoir of the body and plays a central role in nitrogen and protein metabolism. The many diverse functions are controlled by the substrate concentrations and hormone levels in the blood and by the autonomic hepatic nerves. Hepatic regulation of energy supply and consumption therefore has to cope with these special tasks. Regulation of heart energy turnover, on the other hand, is mainly focused on ATP supply for muscle work. This is reflected in the steady-state phosphorylation potentials of ATP, the capacity of the oxidative metabolism and, consequently, the basal oxygen consumption. All these parameters are lower in liver than in heart tissue.

Regulation of energy metabolism in mitochondria as the "power plants of the cell" is fairly complex. This holds even more for mitochondria in the intact cell and in the intact organ. Main aspects for mitochondria *in vivo* are that, due to the presence of cytosolic energy-consuming processes on the one hand, and ATP producing glycolysis on the other hand, the two extreme steady-state conditions, state 4 and level flow, practically do not exist. Thus, the discussion of what controls respiration is also restricted to steady states occurring in the intact cell. Figure 1 gives an overview on bioenergetics in the intact liver cell.

Additionally, hormonal control of energy metabolism starts with extramitochondrial signal cascades and is thus, in general, confined to intact cells. Further important aspects in the regulation of mitochondrial metabolism *in situ* are intercellular as well as intracellular oxygen gradients and intercellular compartmentation of enzymes involved in pathways with ATP turnover. In view of these facts this article will also

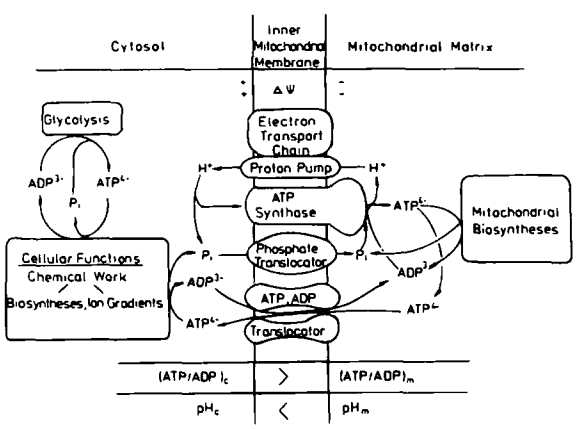

Fig. 1. Energy metabolism in the intact liver cell.

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consider aspects of compartmentation in cellular energy metabolism.

In the last two decades kinetic and thermodynamic models have been developed to quantify the regulation of cellular metabolism. This will be called, according to the suggestions of others, *control* of metabolism. I will not extensively review the immense work done in this field but only discuss the work dealing with the regulation of energy metabolism of intact liver cells and liver.

INTACT LIVER VERSUS ISOLATED MITOCHONDRIA

Zonal Organization of Hepatic Energy Metabolism

The functional units of the liver are the acini which extend from the terminal portal venule along the sinusoids to the terminal hepatic venule. This anatomical zonation causes a functional zonation defined mainly by corresponding oxygen gradients (see below). The functional zonation is manifested in different contents of enzymes, belonging to pathways concerning nitrogen (Häussinger, 1990) and carbohydrate (Jungermann and Katz, 1982) metabolism, along the gradients. Consequently, energy metabolism in the liver is also subject to zonation. Energy-consuming pathways like urea synthesis and gluconeogenesis are predominant or exclusively present in the periportal area of the liver with high oxygen concentration, whereas glycolysis is active in the perivenous area with respective lower oxygen supply. Functional zonation increases tolerance of the liver to oxygen deficiency, restricting pathways with high rates of oxygen consumption to areas with high oxygen supply. Therefore it is not surprising that, despite intraacinar oxygen gradients, gluconeogenesis occurs in perfused liver at the same high rate as in isolated hepatocytes (Krebs *et al.*, 1974). Another advantage of functional zonation is prevention of futile cycles, e.g., in carbohydrate metabolism: glycolysis is suppressed in periportal areas where gluconeogenesis is active and vice versa. Futile cycles occur at considerable rates in isolated hepatocytes where functional zonation is absent (Philips *et al.*, 1995; Newsholme and Parry-Billings, 1992).

On the other hand, oxygen-dependent detoxication reactions, e.g., ethanol oxidation, are not subject to functional zonation and therefore can lead to considerable hypoxic damage (Ji *et al.*, 1980).

Oxygenation of Cell and Intact Organ

In the normoxic liver cell 70–100% of the total ATP is supplied by oxidative phosphorylation. Oxygen is one substrate for the respiratory chain. Since the K_m of oxygen for respiration is below 1 μM (Bienfait *et al.*, 1975), it was believed that mitochondrial oxygen supply is saturating and therefore of no regulatory significance. However, oxygen gradients between the extracellular space and the mitochondria can be up to two orders of magnitude in hepatocytes, depending on the cellular oxygen consumption rate (Jones *et al.*, 1982; Kekonen *et al.*, 1987). In the intact liver additional gradients up to a factor of 2 are built up between periportal and perivenous cells within the liver lobule (Ji *et al.*, 1980; Sies, 1977) that, e.g., in liver perfusions, starting from a portal oxygen concentration of about 500 μM in hemoglobin-free, carbogen gassed Krebs–Henseleit bicarbonate buffer, a mitochondrial oxygen concentration of about 2 μM would result in cells situated in the perivenous area.

Further, the different oxygen-dependent systems have different K_m values for oxygen (Jones *et al.*, 1982, Kekonen *et al.*, 1987; Sies, 1977). Thus, it has been reported that the cellular redox and phosphorylation states are affected by oxygen concentrations up to 100 μM (Wilson *et al.*, 1979; Jones *et al.*, 1982), whereas oxygen consumption is concentration-independent above a low limit (~ 1 μM). Likewise, when increasing the pO_2 of isolated hepatocyte suspensions from 1 to 10 mm Hg, oxygen uptake is stimulated 1.3-fold, whereas the cellular ATP/ADP ratio is raised 2.3-fold (Hummerich *et al.*, 1988). Taken together, oxygen concentration in mitochondria in perivenous cells is presumably within the limiting range for oxidative phosphorylation.

Regulatory counterbalancing of hypoxia or ischemia is probably not specific for liver. A main mechanism is change in affinity of respiration for oxygen. It increases at low pO_2. Kinetic studies indicate that the cause is a change in the reaction constants for electron transfer at cytochrome oxidase redox centers (Bienfait *et al.*, 1975; Sugano *et al.*, 1974, Chan and Li, 1990) which are involved in proton pumping. In isolated rat liver mitochondria a steep dependence of the protonmotive force on the rate of respiration is only observed for very low respiration rates (Westerhoff *et al.*, 1987). Therefore, it is feasible that at low pO_2 changes in protonmotive force might trigger these effects.

From studies using kidney cells it has been proposed that mitochondria compensate low pO_2 by an appropriate decrease in the phosphorylation potential and progressive reduction of cytochrome *c*, thereby keeping respiration constant in a certain range of low pO_2 (Wilson *et al.*, 1977). Whereas the author ascribes this phenomenon to the near-equilibrium of the first two sites of the respiratory chain, it could be due to the fact that the efficiency of oxidative phosphorylation is decreased at low oxygen concentration by partial uncoupling of electron transport and proton pumping (Kramer and Pearlstein, 1983) and that a low phosphate potential causes reduction of cytochrome *c* (Bienfait *et al.*, 1975), thus establishing a new steady state.

Regulation of respiration at low oxygen tension by the phosphorylation potential has the advantage that it is possible even when the absolute amount of adenine nucleotides falls. Irreversible damage of the liver cell occurs when the adenine nucleotide content decreases below 30% (Kobayashi *et al.*, 1990). The preservation of the adenine nucleotide pool is therefore a further critical step in the prevention of hepatic hypoxic damage. Here the regulation of AMP breakdown by AMP-desaminase is of central importance (Vincent *et al.*, 1982). The shift of mitochondrial adenine nucleotides to AMP during hypoxia has been suggested to prevent loss of adenine nucleotides by the purine catabolism in the cytosol, since AMP cannot leave the mitochondria (Dransfield and Aprille, 1994).

Mitochondrial and Cytosolic Phosphorylation States of ATP

Since the inner mitochondrial membrane is impermeable to adenine nucleotides, mitochondria require a specific ATP/ADP exchange system. The adenine nucleotide translocase corresponds finely to the energetic requirements of the cell; as a result of its control by the mitochondrial membrane potential, it is asymmetric in actively respiring mitochondria (for a review see Klingenberg and Heldt, 1982), so that in energized cells the mitochondrial ATP/ADP ratio is always lower than the cytosolic ratio (Fig. 1, Table I). Other nucleotides such as AMP, GTP, GDP, CTP, or CDP are connected to the ATP/ADP system via phosphate transfer reactions in both compartments, poising the other nucleotide systems to the same phosphorylation level as the adenine nucleotide system (Söling, 1982). So far no transport systems for these compounds are known, so that communication of other nucleotides between the two compartments is only possible via the ATP/ADP system.

In Table I ATP/ADP ratios are compared for isolated hepatocytes, perfused liver, and liver *in vivo* and for different rat tissues. Mitochondrial ATP/ADP ratios are lower in hemoglobin-free perfused liver than in liver *in vivo* and in hepatocytes at practically unchanged cytosolic ratios. The lowering of the mitochondrial ratio was ascribed to the lower oxygen tension in the intact organ (see above), whereas the cytosolic ratio is maintained by glycolysis (Soboll *et al.*, 1978). With respect to this finding it is interesting to note that in the intact organs, e.g., the perfused liver, a defined relationship between the mitochondrial ATP/ADP ratio and respiration is not found, in contrast to observations in isolated mitochondria (Duszynski *et al.*, 1981). Therefore, the mitochondrial ATP/ADP ratio does not regulate respiration in intact cells.

Consistent with the finding of lower mitochondrial ATP/ADP ratios in perfused liver than in hepatocytes is the observation that gluconeogenesis is similar in hepatocytes and perfused liver (Table II) whereas urea synthesis, which is very sensitive to the mitochondrial ATP/ADP ratio (Williamson *et al.*, 1981), can reach 3-fold higher rates in hepatocytes (Krebs *et al.*, 1974).

The difference between the mitochondrial and cytosolic phosphorylation potentials of ATP, however, is very similar in hepatocytes and intact liver; the variation in ATP/ADP ratios is balanced by changes in phosphate. The same difference is also observed in other tissues, where the absolute values for mitochondrial and cytosolic ATP/ADP ratios are much higher, e.g., in heart. A constant $\Delta(\Delta G_p)_{(m\text{-}c)}$[2] would be reasonable, assuming that adenine nucleotide and phosphate transport are sufficiently fast, since the energy for adenine nucleotide and phosphate transport should not change. However, there are remarkable differences in $\Delta(\Delta G_p)_{(m\text{-}c)}$ for the fed and fasted state of the liver. In livers from fasted rats glycolysis is absent and adenine nucleotide exchange is affected by the increase in long-chain fatty acyl CoA, both causing the displacement

[2] Abbreviations: $\Delta(\Delta G_{p(m\text{-}c)})$, mitochondrial/cytosolic difference of phosphorylation potentials of ATP; $\Delta pH_{m\text{-}c}$, mitochondrial/cytosolic pH difference; $\Delta\Psi$, membrane potential; pm, plasma membrane; pmf, protonmotive force; X_p, phosphorylation potential of ATP; X_o, redox potential of NAD^+; η, efficiency; J_o, rate of respiration; J_p, rate of ATP synthesis; T_3, T_2, L-triiodothyronine, L-3,5-diiodothyronine; DMO, 5,5-dimethyl-2-4-oxazolidinedione; $TPMP^+$, triphenylmethylposphonium bromide.

Table I. Comparison of Mitochondrial and Cytosolic ATP/ADP Ratios and Difference in Phosphorylation Potentials $\Delta(\Delta G_p)_{(m-c)}$ in Liver Preparations and Other Tissues from the Rat

		ATP/ADP		ATP/ADP	Pi (mM)		$\Delta(\Delta G_p)_{(m-c)}$
		M	C	C[e]	M	C	(kJ/mol)
Rat							
Isolated hepatocytes,	fed[g,h]	2.1	5–12				
	fasted[a]	1.8	8.8		16.8	3.3	8.3
Perfused liver,[b]	fed	0.24	5.3		7.3	6.5	8.3
	fasted	0.67	2.6		8.7	9.5	3.3
Liver *in vivo*,[c]	fed	0.85	7.0		10.0	4.2	7.8
	fasted	1.0	5.9				
Skeletal muscle *in vivo*[d]		2.8	13.2	196	13.0	26.0	9.3
Heart[f]		2.7	5.5	40	4.8	3.7	7.7

[a] From Akerboom *et al.*, 1978; [b] Soboll *et al.*, 1978; [c] Schwenke *et al.*, 1981; [d] Hebisch *et al.*, 1982; [e] free ATP/ADP ratios calculated from creatine kinase equilibrium; [f] Kauppinen *et al.*, 1980; [g] Soboll *et al.*, 1980b; [h] Siess *et al.*, 1977.

of the cytosolic ATP/ADP ratio from equilibrium. Glycolysis appears to be essential for the establishment of a high cytosolic phosphorylation potential in liver. It can be lowered in livers from fed rats by infusion of lactate and pyruvate, which depresses glycolysis, and it can be increased in the fasted state by infusion with glucose (Thurman and Scholz, 1977; Soboll *et al.*, 1978). Wanders *et al.* (1991) have shown in liver mitochondria that adenine nucleotide translocation is displaced from equilibrium already at 30% of state 3 respiration which could be close to the situation in the intact cell (see below). Calculation of the mitochondrial membrane potential from the ratio of mitochondrial/cytosolic ATP/ADP ratios showed that only at low biosynthetic activity and high rate of glycolysis, e.g., in the fed state in the presence of glycogen and glucose, might the translocase reach near equilibrium in liver (Soboll *et al.*, 1980a). Thus, at least for liver mitochondria, experimental data do not support thermodynamic equilibrium for the first two phosphorylating sites of the respiratory chain as postulated by (Wilson *et al.*, 1974).

Especially in heart and skeletal muscle there is some uncertainty in the determination of the cytosolic

Table II. Influence of Metabolic Pathways on Hepatic Oxygen Consumption[a]

	% of total oxygen consumption	
Mitochondrial independent Respiration	14–23 (h)	Brand *et al.*, 1994
Proton leak	20–30 (l,h)	Brown, 1992; Berry *et al.*, 1988; Brand *et al.*, 1994
Na^+ export	45 (l,h)	Whitman, 1974
Protein synthesis	12 (l)	Brown, 1992
	% change in oxygen consumption	
FCCP	+60 to +100 (l,h)	Krebs *et al.*, 1974; Gregory and Berry, 1991
DNP	+80 to +100 (l,h)	Soboll *et al.*, 1978
Amytal	−60 (l)	Brauser *et al.*, 1972
Cyanide	−80 (l)	Brauser *et al.*, 1972
Carboxyatractyloside	−60 (h)	Gregory and Berry, 1991
Aminooxyacetate (protein turnover)	−44 (l)	Thurman and Scholz, 1977
Gluconeogenesis	+60 (l,h)	Krebs *et al.*, 1974
Urea synthesis	+100 (l) to +360 (h)	Krebs *et al.*, 1974
Glycolysis	−5 to −55 (l)	Soboll *et al.*, 1978

[a] (l) liver; (h) hepatocytes.

phosphorylation potential because of possible binding of ADP and phosphate to cytosolic proteins. With the help of the creatine kinase reaction the free cytosolic ATP/ADP ratio in heart and muscle tissue can be calculated, since this enzyme is in equilibrium under most circumstances (Veech *et al.*, 1979). However, in the liver the creatine kinase is absent and other enzyme systems involving ATP, like the glyceraldehyde-3-phosphate/phosphoglycerate kinase system (van der Meer *et al.*, 1978), are not as reliable as the creatine kinase system. Two studies using isolated liver cytosol (Mörikofer-Zwez and Walter, 1989) or permeabilized rat liver cells (Gankema *et al.*, 1983) showed that maximally 30–50% of ADP could be bound in rat liver cytosol. This would not change the cytosolic phosphorylation potential substantially. ^{31}P nuclear magnetic resonance spectroscopy indicates binding of ADP and phosphate in liver (Iles *et al.*, 1985) in contrast to (Gankema *et al.*, 1983) who did not find cytosolic binding of phosphate. ^{31}P NMR, however, has a relatively low sensitivity for phosphorus compounds and thus, until now, it was not possible to measure reliably the cytosolic phosphorylation potential.

The Mitochondrial Steady State in the Liver Cell

In mitochondria *in situ* the redox state of the mitochondrial NAD system is remarkably different from isolated mitochondria. The mitochondrial redox potential in perfused livers from fed rats, as deduced from the titration of the perfusate hydroxybutyrate/acetoacetate ratio, has been shown to be −294 mV at 37° and pH 7.0, corresponding to a $NAD^+/NADH$ ratio of 1–2 (Sies, 1982); similar values are found for hepatocytes (Wilson *et al.*, 1974). In isolated liver mitochondria, on the other hand, the $NAD^+/NADH$ ratio is 10- to 100-fold smaller (Wilson *et al.*, 1974) and the ATP/ADP ratio established in the medium by isolated liver mitochondria is much higher (around 100) than the values found in the intact liver cell. This is in line with the finding of a fairly constant mass action ratio for the first two sites of the respiratory chain and the cytosolic phosphorylation potential by Wilson (accordingly, the cytosolic ATP/ADP must increase when NAD^+ becomes more reduced; Wilson *et al.*, 1974), although his conclusion, that these reactions are in near equilibrium, must be questioned (see above).

In addition, mitochondrial steady states in intact cells and organs depend on the nutritional state. From their redox pattern and phosphorylation state, livers from fed rats appear to be close to state 4 of coupled mitochondria *in vitro*, i.e., substrate is saturating and ADP is low (Brauser *et al.*, 1972; Krebs *et al.*, 1974). In substrate-free perfused livers from fasted rats mitochondria are substrate limited, corresponding to state 2, and in the presence of substrates for gluconeogenesis or urea synthesis (high substrates, high ADP), mitochondria should be close to state 3. The corresponding respiratory rates, however, are fundamentally different from isolated mitochondria: Livers from fed rats in the resting state (close to state 4) have a high basal respiration rate of about 120–150 μmol/g · h (Soboll *et al.*, 1978). Similar rates are obtained for hepatocytes (Krebs *et al.*, 1974). This rate is about the same as in substrate-free perfused liver from fasted rats (substrate limited state). In both states (i.e., states 4 and 2) respiration of isolated mitochondria would be close to zero.

On the other hand, stimulation of respiration by substrates (gluconeogenesis, urea synthesis) or ADP (uncoupler) increases oxygen uptake maximally by 50–300% (Table II) whereas in isolated mitochondria it is increased up to 10-fold. This is due to the high basal respiration in intact cells, where up to 40% accounts for mitochondrial independent respiration + proton leak, and up to 40% for sodium export (Table II). Another important factor, especially in substrate-free perfused livers from fasted rats, is protein turnover. Protein turnover in the rat accounts for 20% of the basal metabolic rate (Waterlow, 1984). The liver is responsible for about 1/4 of total protein synthesis, so its share on overall protein turnover should be high. Inhibition of transaminases by aminooxyacetate decreases respiration by 40% in perfused liver (Table II), which could be a rough estimate of energy consumed by proteolysis and protein synthesis in the isolated organ. It should be lower in liver *in vivo*, where the extracellular supply with amino acids and other substrates depresses protein turnover. Respiration in the intact liver cell is also dependent on glycolysis. There is an inverse relationship between both parameters (Thurman and Scholz, 1977). Consequently, in livers from fed rats, basal respiration is lower due to inhibition by glycolysis (Table II).

The *mitochondrial* parameters responsible for the high basal respiration in the intact liver cell are proton leak and slip in the redox and ATPase proton pumps. Proton leak has been shown to be high in the resting state and low in active respiring isolated mitochondria (Brown 1992; Brand *et al.*, 1994), whereas slip is high at a high turnover of the proton pumps, i.e., in actively respiring mitochondria (Groen *et al.*, 1990; Luvisetto

et al., 1992). The share of proton leak to basal respiration has been estimated to be in the range of 20–30% in the intact cell (Brand *et al.*, 1994), but no estimation about the share of slip to basal respiration in the intact cell is available. According to Brand *et al.* (1994) slip is not very large. This, however, appears not realistic in view of inhibitor titration studies in isolated mitochondria (e.g., Pietrobon *et al.*, 1983; Luvisetto *et al.*, 1992). Of special interest is the question of the contribution of slip or leak to the increase in basal respiration in the hyperthyroid state (see below).

METABOLIC AND HORMONAL REGULATION OF ENERGY METABOLISM

Influence of Fatty Acid Metabolites

Fatty acid metabolites have a great impact on the energy metabolism, since, besides their role as metabolic fuels, high levels of free fatty acids uncouple oxidative phosphorylation (e.g., Lehninger and Remmer, 1959) and their coenzyme A derivatives inhibit mitochondrial adenine nucleotide transport (Shug *et al.*, 1971) in isolated mitochondria. The physiological relevance of these actions has been questioned, based mainly on the observation that addition of fatty acids to hepatocytes causes not only an increase in oxygen consumption but also of the phosphorylation state of the cell. Further, the stimulation of respiration by fatty acids is oligomycin sensitive (see Brand and Murphy, 1987). The influence of fatty acids on the liver cell, however, depends critically on the experimental condition. Fatty acids caused an increase in oxygen consumption in hemoglobin-free perfused liver and, depending on the concentration of free fatty acids, either an increase or a decrease in mitochondrial and cytosolic phosphorylation states of ATP (Soboll *et al.*, 1984a). β-Oxidation of fatty acids greatly enhances the supply of reducing equivalents to the respiratory chain and therefore stimulates ATP synthesis; this is reflected in a higher phosphorylation state of the liver. On the other hand, when raising the free fatty acid level, they cause uncoupling of oxidative phosphorylation; therefore, the phosphorylation state of the liver decreases. Thus, uncoupling by fatty acids only plays a role when the oxidative capacity of the organ is not high enough to prevent accumulation of free fatty acids, e.g., in the liver in zones with low oxygen concentrations (Soboll *et al.*, 1984a). Kinetic investigation of the increase in oxygen consumption in perfused liver revealed two components; one was ascribed to the stimulation of oxidative phosphorylation and the other to the increase in respiration due to uncoupling (Scholz *et al.*, 1984). The inhibitory effect of oligomycin on fatty acid stimulated respiration is also dependent on the amount of free fatty acids present. At high free fatty acids, oligomycin cannot abolish stimulation of respiration in hepatocytes (own observations). Several models for the mechanism of uncoupling by free fatty acids were discussed. A classical protonophoric action of long-chain fatty acids is questionable due to the slow mobility through the lipid bilayer. Rottenberg (1990) classifies fatty acids as decouplers, agents which stimulate respiration and inhibit ATPsynthesis, without a decrease in protonmotive force, by affecting the intramembranal proton transfer pathway.

During fasting and diabetes the concentrations of unesterified fatty acids are high in the blood and high rates of fatty acid oxidation and ketogenesis are observed in the liver. Ketone bodies are supplied to the organism as substrate. It appears therefore reasonable under conditions of exclusive fatty acid oxidation that this process is not restricted by the energy needs of the liver cell (Scholz *et al.*, 1984). A recent study using cytochrome oxidase reconstituted in liposomes demonstrated that fatty acids translocate protons only above a threshold membrane potential above 125 mV. Accordingly, ATP synthesis is not inhibited by the uncoupling effect up to a certain level, above which oxidation of substrates takes place independently from phosphorylation (Kröhnke *et al.*, 1993). This would be in good agreement with the arguments raised above.

When adding carboxyatractyloside, an inhibitor of mitochondrial adenine nucleotide translocation, to perfused livers, the mitochondrial ATP/ADP ratio increases and the cytosolic ratio decreases (Soboll *et al.*, 1978). The same is observed in livers from fasted rats (Soboll *et al.*, 1984b). In these livers also the content of long-chain fatty acyl CoA is increased. The effect on both adenine nucleotides and fatty acylCoA is reversed on refeeding. The changes observed can be mimicked when infusing livers from fed rats with oleate, a long-chain fatty acid, but not with octanoate, a short-chain fatty acid. Inhibition of adenine nucleotide transport by long-chain fatty acylCoA could be of metabolic relevance in the intact cell: The increase in the mitochondrial phosphorylation state might be of importance for gluconeogenesis and, especially, urea synthesis; both are dependent on the mitochondrial

phosphorylation state and are pronounced during fasting (Soboll *et al*, 1984b).

Regulation of Hepatic Energy Metabolism by Short-Term Acting Hormones

During stress or starvation the liver has to supply the organism with glucose as respiratory or glycolytic substrate. The switch to a glucogenic situation is controlled by the so-called "glucogenic hormones" which all stimulate respiration, gluconeogenesis, and urea synthesis in the liver, although through different signaling pathways (for a review see Soboll and Sies, 1989). To this group belong glucagon, vasopressin, α-adrenergic agonists, and thyroid hormones. The time courses of changes of the three pathways have been shown to be similar, but specific for each hormone (Hummerich and Soboll, 1989). The common mediator of these changes might be calcium, since the changes in extra- and intracellular calcium elicited by glucogenic hormones, including cAMP-dependent hormones like glucagon, follow the same time course.

Gluconeogenesis

Gluconeogenesis is enhanced through inhibition of pyruvate kinase by phosphorylation of the enzyme (see, e.g., a review by Soboll and Sies, 1989), and through an increase in flux through pyruvate carboxylase (Agius and Alberti, 1985). The mechanism of activation of pyruvate carboxylase is unclear, since the mitochondrial ATP/ADP ratio is not increased with, e.g., glucagon and T3 (see below), but might be due to the enhancement of the acetyl CoA/CoA ratio during fatty acid oxidation in the fasted state.

Urea Synthesis

Likewise urea synthesis, which is very sensitive to the intramitochondrial phosphorylation state, appears not to be regulated via the latter by glucogenic hormones. The activation of *N*-acetylglutamate synthetase by micromolar calcium (Johnston and Brand, 1990) and also the stimulation of glutaminase (Joseph and McGivan, 1978) could be responsible for the stimulation of urea synthesis.

Respiration

The intramitochondrial redox state of NAD^+ becomes more reduced through the action of calcium on mitochondrial dehydrogenases (McCormack *et al.*, 1990). This increase in substrate supply to the respiratory chain is supposed to be the main basis for an increase in respiration by glucogenic hormones. Changes in cellular ATP appear secondary as effectors in view of the fact that stimulation of respiration persists when mitochondria are isolated after hormone treatment of the liver. On the other hand, this conclusion has been questioned as an artefact of the isolation procedure of the mitochondria (Siess, 1983). Furthermore, in perfused liver from fasted rats respiration is only stimulated by glucagon in the presence of substrates for gluconeogenesis, whereas in livers from fed rats the stimulation of respiration has been assigned to the inhibition of glycolysis at the pyruvate kinase step (Kimmig *et al.*, 1983). These findings would be in good agreement with the proposal that, in addition to the increase in substrate supply via calcium-sensitive dehydrogenases, it is also the change in ATP turnover induced by glucogenic hormones which regulates respiration.

The question is, if at all, glucogenic hormones cause changes in the phosphorylation states of ATP, the membrane potentials, and the protonmotive force in the intact cell and if the effects are the same for all glucogenic hormones. Table III gives an example of the way glucagon, T_3, and vasopressin influence these parameters in perfused liver. The picture is different for each hormone: whereas glucagon and T_3 increase $\Delta pH_{m\text{-}c}$ and pmf, only glucagon increases also $\Delta\Psi_{m\text{-}c}$ and only T_3 increases $\Delta\Psi_{pm}$. Vasopressin has no effect on any of the parameters. Likewise, in other studies, it is shown that glucagon has no influence on mitochondrial ATP/ADP in the fed and in the fasted liver (Soboll and Scholz, 1986; Siess *et al.*, 1977), decreases the cytosolic ratio in the fed state and increases it in the fasted state (Soboll and Scholz, 1986), whereas T_3 and adrenaline increase the mitochondrial ratio and decrease the cytosolic ratio (Soboll and Scholz, 1986; Seitz *et al.*, 1985). It is clear from these examples that, although the metabolic responses might be similar for glucogenic hormones in liver, the steps, where control is exerted, are different. Measuring the steady-state parameters of cellular energy state is therefore of limited value for the investigation of regulation of liver energy metabolism by glucogenic hormones, since too many reactions can influence these parameters. Control

Table III. Influence of Glucogenic Hormones on $\Delta\Psi$, ΔpH_{m-c}, and pmf in Isolated Perfused Rat Liver[a]

Condition	$\Delta\Psi m$ (mV)	$\Delta\Psi pm$ (mV)	ΔpH_{m-c}	pmf (mV)
Fasted, lactate + pyruvate	144 ± 1	63.4 ± 5	0.23 ± 0.005	158 ± 4
+L-triiodothyronine, 10^{-6} M	142 ± 1	80.0 ± 5[b]	0.61 ± 0.07[b]	179 ± 5[b]
+glucagon, 10^{-8} M	160 ± 4[b]	56.0 ± 9	0.50 ± 0.07[b]	191 ± 8[b]
+vasopressin, 10^{-8} M	145 ± 3	56.3 ± 5	0.25 ± 0.07	160 ± 6

[a] Mean values ± SEM, from Soboll (1993a).
[b] $p < 0.05$ compared with control.

analysis might be a more promising theoretical and practical approach (see below).

Influence of the Thyroid Status on Hepatic Energy Metabolism

Thyroid hormones regulate the basal metabolic rate in mammals and, especially in the liver, the metabolism of every class of metabolic substrate. Related to these functions is their predominant role in thermogenesis. They affect cellular energetics mainly by regulation of gene expression, but in recent years growing evidence was presented that T_3 and T_2 also have short-term glucogenic effects in the liver via an increase in calcium influx into the cell (for a review see Soboll, 1993b). In isolated mitochondria high doses of T_3 ($>10^{-5}$ M) cause uncoupling of oxidative phosphorylation. Therefore, it was supposed that the stimulation of respiration seen in hyperthyroid mammals might be due to uncoupling. However, the increase in oxygen uptake elicited by T_3 in perfused liver is oligomycin sensitive and gluconeogenesis and urea synthesis are increased (Müller and Seitz, 1980), strong arguments against uncoupling.

The enhanced basal rate of oxygen consumption in the hyperthyroid liver can be assigned to the higher amount of respiratory chain proteins, to induction of Na^+/K^+-ATPase, and to an increase in futile cycling (for a review see Soboll, 1993b). Further, already very early it was shown that ADP uptake is stimulated by thyroid hormones in isolated mitochondria (Hoch, 1977). That adenine nucleotide translocation is stimulated was also deduced from the finding that higher concentrations of carboxyatractyloside were necessary for inhibition in hepatocytes in higher thyroid states (Gregory and Berry, 1991) and from a decrease in the mitochondrial ATP/ADP ratio and a corresponding increase in the cytosolic ratio in perfused rat liver and rat liver *in vivo* (Seitz *et al.*, 1985). Changes in lipid composition of the inner mitochondrial membrane and/or enhanced expression of an adenine nucleotide translocase gene are discussed as possible mechanisms (see Soboll, 1993b).

Using control analysis, Harper and Brand (1993) measured in hepatocytes, that in the euthyroid compared to the hyperthyroid state 50% of the increase in oxygen consumption was accounted for by mitochondrial independent oxygen consumption and 50% was due to increase in proton leak; in the hyperthyroid state again 50% was accounted for by proton leak and the remaining 50% was due to the increase in cellular ATP turnover. On the other hand, in isolated mitochondria from T_3-treated rats, using inhibitor titrations of the proton pumps Luvisetto *et al.* (1992) showed that the increase in respiration compared to control mitochondria was due to slipping of the proton pumps. This interpretation would be more reconcilable with the induction of electron transport chain and H^+-ATPase proteins (i.e., more pumps) in hyperthyroidism (for a review see Soboll, 1993b).

Can the decrease in efficiency observed in higher thyroids states, like the effect of free fatty acids, be regarded as "physiological uncoupling" (independent of a slip or leak mechanism)? "Physiological" means that it does not prevent enhanced ATP supply for cellular work. The advantage would be a relief of the strong restriction of respiration to the ATP needs of the cell (i) to produce heat in higher thyroid states, or (ii) to maintain a favorable mitochondrial redox state of NAD^+ during fatty acid oxidation and to generate ketone bodies for peripheral organs during fasting.

CONTROL OF ENERGY METABOLISM

Oxidative phosphorylation consists of an oxidation and a phosphorylation system. The oxidative sys-

tem comprises substrate supply, i.e., transport of oxygen and of oxidizable substrates, and the redox systems of the respiratory chain. The phosphorylation system comprises the ATP-synthase and the ATP/ADP and phosphate carriers. Both systems are coupled through the protonmotive force built up by the proton pumps of the respiratory chain and used by the H^+-ATPase (Fig. 1).

Is respiration controlled by ATP consumption or ATP production? This leads to the next question: What controls ATP production and what controls ATP consumption? Finally, we must decide which model we choose when describing control. Description started with kinetic considerations, i.e., the question which reaction step limits a pathway. However, *in vivo* metabolic conditions change; the liver is controlled by the substrates and hormones coming with the bloodstream and determining its metabolic state, and thus control of respiration might change between several steps. These thoughts have led to the application of the theory of metabolic control, developed by Kacser and Burns (1973) and Heinrich and Rapoport (1974), to oxidative phosporylation (Groen *et al.*, 1982). According to this treatment, control coefficients tell that, e.g., a certain change in the level of a parameter *A* will cause a corresponding change in a parameter *B*. If choices for parameter *A* are limited to enzymes, all control coefficients for the flux through a metabolic pathway add up to one. Therefore, parameters having flux control coefficients in the range between 0.5 and 1.0 exert high control.

On the other hand, biological systems also have to follow physical constraints fixed in the thermodynamic laws. It might be useful to consider which thermodynamic constraints oxidative phosphorylation has to follow. This has been examined in the irreversible thermodynamic treatment of oxidative phosphorylation (Caplan and Essig, 1969; Walz, 1990). The rate of oxygen consumption, the flux J_o, was found to be a phenomenologically linear function of X_p, $\Delta\Psi_m$, or pmf in phosphorylating systems, which made experimental and mathematical treatment of oxidative phosphorylation by this formalism practicable. Linear flow–force relationships were also found in the intact hepatocyte (van der Meer *et al.*, 1978; Berry *et al.*, 1988). The disadvantage of this treatment is that information, e.g., on the influence of the single components on the system cannot be obtained. The gap between the nonequilibrium thermodynamic treatment and the actual mechanisms occurring was bridged in the mosaic nonequilibrium thermodynamic description of oxidative phosphorylation which represents a combination of kinetic and thermodynamic treatment (Van Dam and Westerhoff, 1977). The whole system is divided into several blocks, where the uninformative phenomenological coefficients are expressed in terms of the rate constants of the chemical reactions and transport processes.

Both models, control analysis and near-equilibrium thermodynamic treatment, have produced a wealth of experimental work and results and gave new insights into the regulation of energy metabolism. The last two paragraphs briefly summarize the main outcome of this work for intact cells (for reviews see also Brand and Murphy, 1987; Moreno-Sanchez and Torres-Marquez, 1991; Brown, 1992).

Control of Oxidative Phosphorylation in Liver Cells and Liver

The main result is that control is shared between several reactions and that control there varies, i.e., reactions sometimes exert much control and sometimes they do less.

Substrate Supply, Proton Leak, and Cytosolic Phosphorylation Potential

In the liver respiration can be predicted reasonably from the NADH/NAD ratio, the cytosolic phosphorylation potential, and oxygen concentration. This is reasonable in view of fairly high control coefficients over respiration reported for reactions that supply NADH to the electron transport chain (0.15–0.3; Brown *et al.*, 1990; Harper and Brand, 1993) and for adenine nucleotide transport (0.3, Duszynski *et al.*, 1982) in hepatocytes. Control coefficients of 0.3 for electron transport chain reactions, 0.5 for ATP synthesis, transport, and consumption, and 0.2 for proton leak were determined in the fed and fasted hepatocyte (Brown *et al.*, 1990). This might be different, however, for the intact liver in the fed and fasted state, since subcellular ATP/ADP ratios are unchanged in the fed and fasted hepatocytes but not in intact liver (Table I).

Since a value of about 0.3 has been determined for adenine nucleotide transport in hepatocytes (Duszynski *et al.*, 1982), only little control remains for ATP synthesis and consumption (0.2). On the other hand, ATP transport and consumption have a high control over ATP synthesis (0.84), whereas electron transport

chain has a control coefficient of 0.23 over this process (Brown *et al.*, 1990).

Control of respiration by the cytosolic phosphorylation potential in liver implies not only control by adenine nucleotide transport. In certain cases transport of phosphate can become limiting despite the very high capacity of the mitochondrial phosphate carrier. In a ^{31}P NMR study using isolated perfused liver in combined urea synthesis and gluconeogenesis, a Michaelis–Menten type dependence of respiration on cytosolic phosphate concentration was demonstrated (Tanaka *et al.*, 1989).

Using the top-down approach of metabolic control analysis in isolated hepatocytes, control coefficients of 0.3, −0.34, and 0.03 of phosphorylation reactions, proton leak, and substrate oxidation reactions, respectively, over the P/O ratio were determined. This showed that in the intact cell proton leak and phosphorylation reactions have a high control over efficiency of oxidative phosphorylation in contrast to substrate oxidation reactions (Brand *et al.*, 1993).

Modulation of control coefficients over respiration by glucogenic hormones appears to occur mainly at the site of NADH supply via a rise in intramitochondrial calcium (control of substrate supply over respiration) and at the site of ATP consumption via activation–deactivation of enzymes, e.g., activation of *N*-acetylglutamate synthase and glutaminase for urea synthesis or activation of pyruvate carboxylase and inhibition of pyruvate kinase for gluconeogenesis (see above). Thus, control over gluconeogenesis shifts from respiration (substrate supply) in the absence, to pyruvate carboxylase and pyruvate kinase (ATP turnover) in the presence, of glucagon and phenylephrine (Pryor *et al.*, 1987; Groen *et al.*, 1986). Control of urea synthesis is at the site of carbamoyl phosphate synthesis (ATP supply) in the absence *and* in the presence (ATP and ammonia supply) of glucogenic hormones.

Oxygen Concentration

This aspect is mainly relevant when regarding the intact organ. Oxygen supply can be limiting in perivenous areas of the liver lobule (see above). Besides changes in the affinity for oxygen of cytochrome oxidase (Wilson *et al.*, 1977), oxygen also influences the expression of enzymes, e.g., phosphoenolpyruvate carboxykinase (Nauck *et al.*, 1981), and this is modulated by hormones like glucagon. Control of oxygen over respiration is thus difficult to estimate in the intact organ due to heterogeneity of the cells within the organ.

Efficiency of Hepatic Energy Conversion

Efficiency of oxidative phosphorylation is best described, in terms derived from nonequilibrium thermodynamics for linear energy converters, as the ratio of the ouput power over the input power:

$$\eta = J_p X_p / J_o X_o$$

It is dependent on the degree of coupling q between electron transport and mitochondrial ATP synthesis (for a description see Stucki, 1980a). Using this formalism, it was postulated that a degree of coupling of 1, i.e., complete coupling, is not compatible with high efficiency, since it leads the system to a completely unphysiological situation where the flux approaches zero. Indeed this situation is not observed in the intact liver cell. Consequently the cell should have means to regulate the degree of coupling and adapt it to the work load of the cell, so that it could convert redox energy into the chemical energy of ATP at optimum efficiency. It could be shown that in liver degrees of coupling are adjusted which allow the cell to operate at optimal efficiency for maximal output flow in the fasted state and maximal output power in the fed state, respectively, and that coupling is modulated by fatty acids (Soboll and Stucki, 1985).

Changes in efficiency of oxidative phosphorylation by modulating coupling mainly affect the cytosolic phosphorylation potential. Therefore, regulation of the cytosolic phosphorylation potential is of critical importance in this concept. Stucki (1980b) showed that buffering of changes in the cytosolic phosphorylation potential is achieved by the so-called thermodynamic buffer enzymes, kinases which keep cytosolic ATP constant at the expense of a rise of AMP in liver (adenylate kinase) or creatine in muscle tissue (creatine kinase).

It was, however, questioned, whether it might make sense for the cell to "waste" energy by lowering the degree of coupling instead of reducing simply the flow, when a high phosphorylation state of the cell is reached (Gnaiger, 1989). However, in terms of regulation, it is easier to adjust a flow, than to slow a system down and then stimulate it again. This has been pointed out by Brand, when discussing the use of proton leak (Brand *et al.*, 1994). Nature has given the answer. It

modulates coupling by introducing proton slip and/or leak, e.g., through thyroid hormones or through free fatty acids. At least for liver, this concept appears to work.

By focusing on the question of "how efficient is a high cytosolic phosphorylation potential or a high rate of ATP synthesis" rather than on "how it is controlled," as does control theory, the thermodynamic concept raised an additional important aspect for the regulation of biological energy conversion.

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