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Regulation of the degree of coupling of oxidative phosphorylation in intact rat liver

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The degree of coupling of oxidative phopshorylation q was determined in isolated perfused livers and in livers in vivo from fed and fasted rats. This determination of q was based on a simple nonequilibrium-thermodynamic representation of the major reactions of cytosolic adenine nucleotides, and made use of the measured cytosolic concentrations of adenine nucleotides, phosphate, and lactate/pyruvate ratios in extracted livers. The deviations of the measured values from the theoretically predicted ones at different mass action ratios of the adenylate kinase reaction showed that the basic assumptions of the model, including linearity between flows and thermodynamic forces, were fulfilled in intact liver within the experimental error. The degree of coupling was higher in livers from fed rats than in livers from fasted rats. In particular, the determined values of q were close to the theoretical degrees of coupling q_p^{ec} and q_f^{ec} which allow maximization of output power and output flow of oxidative phosphorylation for fed and fasted states, respectively, at optimal efficiency and minimal energy costs. This finding indicates that conductance matching between the load and phosphorylation is fulfilled in vivo. Moreover, it was found that fatty acids lower the degree of coupling in a concentration-dependent manner. This suggested that in livers in the fasted state q is decreased due to elevated fatty-acid levels. Thus fatty acids could act as metabolic regulators of the degree of coupling, enabling the cell to optimize efficiency of oxidative phosphorylation under different metabolic regimes.

Introduction

Classical equilibrium thermodynamics predicts that the highest efficiency of an energy converter can be achieved when the energy-transducing processes are fully coupled and when the system is in a state of thermodynamic equilibrium. Such a device is the well-known Carnot machine. For biological systems this prediction of maximal efficiency is, however, of little use, since the state of thermodynamic equilibrium is characterized by a simultaneous vanishing of all net flows and thermodynamic forces. It is quite clear that an organism could not survive under such conditions and that therefore a definition of efficiency has to be sought which is meaningful for nonequilibrium systems and which considers non-vanishing net flows and forces.

A new approach to a definition of efficiency which is useful for biological systems has been formulated by Kedem and Caplan [1], and is based on the formalism of nonequilibrium thermodynamics. A straightforward application of this theory to oxidative phosphorylation has shown that a fully coupled energy converter is incompatible with optimal efficiency, unless all net flows vanish [2]. In the light of this theory the Carnot machine

Abbreviation: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

appears to be a special state of a general energy converter for the case of thermodynamic equilibrium. The inescapable and intuitively uncomfortable consequence of this result is that biological energy converters cannot be fully coupled if they are supposed to operate at optimal efficiency.

A convenient measure for the coupling of an energy converter has been defined by Kedem and Caplan [1] in terms of a dimensionless number q between 0 and 1, called the degree of coupling. A detailed investigation of different states of energy converters, such as oxidative phosphorylation, has shown that not only $q < 1$, but, in addition, that q can assume several well-defined values depending on the energetic needs of the cell [2]. For example, the maintenance of a maximal output power of oxidative phosphorylation at optimal efficiency and minimal energy costs requires $q_p^{ec} = 0.972$, whereas maximal net flow of ATP synthesis at optimal efficiency and minimal energy costs demands $q_f^{ec} = 0.953$.

In vivo the energy demands of the liver cell, for example, are changed by a transition from a glycolytic to a gluconeogenetic regime. In the former situation the liver is in a metabolic resting state where economic output power is of interest, whereas in the latter state more emphasis is put on an increased net ATP production, and hence here economic net ATP flow should be of main interest. From the above it is clear that consequently also q should change in order to guarantee an optimal efficiency of ATP synthesis upon changes of the metabolic state. In other words, q should not be a constant but a parameter subject to metabolic regulation. The question therefore arises, whether and how the liver cell manages to regulate the degree of coupling of oxidative phosphorylation in response to changes of the energetic demands.

Two facts known from the literature can assist us in solving this problem: (1) free fatty acids can uncouple oxidative phosphoryaltion in isolated mitochondria [3–5], (2) the fatty-acid concentration in the liver increases upon starvation of an animal [6]. Combination of these two facts opens up the possibility that q could be regulated in a manner which is consistent with the energy requirements of the cell. For this reason we investigated whether the degree of coupling of oxidative phosphorylation in the whole liver is changed by different metabolic regimes and whether fatty acids affect the degree of coupling in perfused rat livers.

The next section discusses the supporting theory for the estimation of the degree of coupling from measured cytosolic adenine nucleotide concentrations. In the Methods section the experimental details needed for this study are briefly summarized. The succeeding section presents and discusses the main result, namely the lowering of q by starvation and the possible role of fatty acids as a regulator of this parameter.

Theory

Flux model and steady-state equations of cytosolic adenine nucleotides

In this paper we consider a very simple model of the fluxes which determine the steady-state concentrations of the adenine nucleotides in the cytosol of the liver. Fig. 1a depicts a reaction scheme of the adenine nucleotides characterized

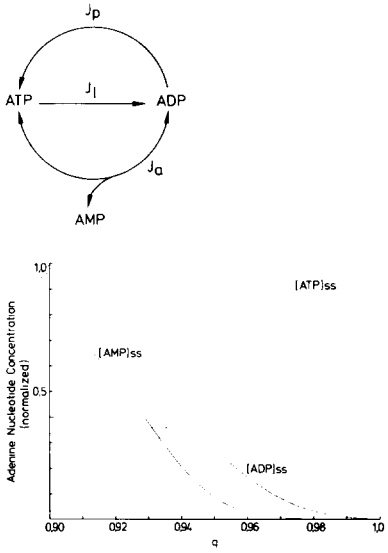

Fig. 1. (a) Flux model of cytosolic adenine nucleotides. The scheme shows how the three different fluxes J_p (phosphorylation), J_l (load) and J_a (adenylate kinase) affect the concentrations of the cytosolic adenine nucleotides (see text). (b) Steady-state solutions of cytosolic adenine nucleotides. The steady-state concentrations of the cytosolic adenine nucleotides normalized by $\Sigma = 1$ are plotted as a function of q based on Eqns. 1–3. The following parameters were used: $X_0 = 184$ kJ, $Z = 2.65$, $\theta = 1$ (see text) and 8 mM P_i.

by three different net flows only: the production of ATP in oxidative phosphorylation (J_p), the utilization of ATP by the cellular energy-requiring reactions, i.e., the load (J_l) and the net flow through the adenylate kinase reaction (J_a). The justification for the omission of additional pathways, notably glycolysis, will be given below.

Apart from this drastic simplification of the major bioenergetic processes in the liver we use an additional fundamental simplification: we assume linear relations between net flows and thermodynamic forces. For isolated mitochondria this linearity has been demonstrated to hold not only at a steady state but also during a transient from one state to another [7]. For the case of whole livers we can obtain information about the validity of the model, including the linearity assumption, by comparing the dependence of a parameter, χ^2, of the system on the mass action ratio of the adenine nucleotides as explained below.

Inspection of Fig. 1a reveals that a steady-state situation is reached whenever there is no net accumulation or utilization of AMP. According to this model this is the case when the adenylate kinase reaction has reached thermodynamic equilibrium. Thus any deviation from a steady-state situation can be detected by comparing the measured mass action ratio with the equilibrium value of the adenylate kinase reaction.

The steady-state equations of our model have been worked out in earlier studies [8]:

$$[\mathrm{ATP}]_{ss} = \frac{\Sigma\, e^{\Psi}}{1+e^{\Phi}+e^{\Psi}} \tag{1}$$

$$[\mathrm{AMP}]_{ss} = (\Sigma - [\mathrm{ATP}]_{ss})\frac{e^{\Phi}}{1+e^{\Phi}} \tag{2}$$

$$[\mathrm{ADP}]_{ss} = \Sigma - [\mathrm{ATP}]_{ss} - [\mathrm{AMP}]_{ss} \tag{3}$$

whereby:

$$\Phi = \frac{\left(\Delta G^0_{phos} - \Delta G^0_{Ak} - RT\ \ln[\mathrm{Pi}]\right)(1+\theta^2) - \frac{qX_0}{Z}}{\left(1+\theta\sqrt{1-q^2}\right)RT}$$

$$\Psi = \frac{\left(RT\ \ln[\mathrm{Pi}] - \Delta G^0_{phos}\right)\left(1+\theta\sqrt{1-q^2}\right) + \frac{qX_0}{Z}}{\left(1+\theta\sqrt{1-q^2}\right)RT}$$

The following parameters appear in these equations: $\Sigma = [\mathrm{ATP}]_{ss} + [\mathrm{ADP}]_{ss} + [\mathrm{AMP}]_{ss}$; the degree of coupling, q; the phenomenological stoichiometry, Z, of oxidative phosphorylation, $\Delta G^0_{phos} = 35.6$, and $\Delta G^0_{Ak} = 0.63$ kJ/mol [2]. The parameter θ is a dimensionless measure for deviation from conductance matching: $L_l = \theta L_p\sqrt{1-q^2}$ with $\theta = 1$, where L_p is the conductance of phosphorylation and L_l the conductance of the load. If $\theta = 1$, we have conductance matching; if $\theta \neq 1$, we do not. Apart from these parameters we have the redox potential of the oxidizable substrates as estimated by the lactate/pyruvate ratios and mean oxygen concentration in the tissue:

$$X_o = \Delta G_{ox} + RT\ \ln\frac{[\mathrm{lactate}]}{[\mathrm{pyruvate}]} + RT\ \ln\sqrt{[\mathrm{O_2}]}$$

with $G^0_{ox} = 194$ kJ/mol [9] and a mean tissue oxygen concentration of 0.035 mM [10].

Hence, in Eqns. 1–3, we are left with the parameters q, Z and θ in order to determine the steady-state concentrations of the adenine nucleotides. Conversely by assuming fixed values for Z and θ, we can estimate q from the measured adenine nucleotide concentrations in the cytosol of whole livers.

Estimation of the degree of coupling of oxidative phosphorylation and test of the assumptions

Fig. 1b shows the steady-state concentrations of the adenine nucleotides plotted as a function of q in the interval $0.9 < q < 1.0$ for fixed values of the parameters θ and Z. Since in this range the steady-state functions depend very sensitively on q, we can expect a rather accurate estimate of this parameter from the measured adenine nucleotides. This estimation is best done by a variation of q such that the deviations of the mesured adenine nucleotides (subscript m) from the solutions of the steady-state equations (subscript ss) become minimal. Thus the objective consists in minimizing the quadratic function of the normalized adenine nucleotide concentrations:

$$\chi^2 = \left\{([\mathrm{ATP}]_{ss} - [\mathrm{ATP}]_m)^2 + ([\mathrm{ADP}]_{ss} - [\mathrm{ADP}]_m)^2 + ([\mathrm{AMP}]_{ss} - [\mathrm{AMP}]_m)^2\right\}(\Sigma^2)^{-1}$$

which has the geometric interpretation of a unit squared distance or Euclidean norm.

In order to carry out this minimization, we have first to assume some values for θ and Z. For Z we can use the average value which has been estimated for whole perfused livers: $Z = 2.65$ [11]. Furthermore, by assuming that for not too short time periods the liver manages to establish conductance matching of the load, we can put $\theta = 1$. Of course this latter value is a mere assumption and there exists unfortunately no independent method for experimentally assessing this quantity. Therefore let us look what happens when θ deviates from unity.

Fig. 2 depicts χ^2 as a function of q for various values of θ. The reference adenine nucleotide concentrations were obtained by setting $q = q_f^{ec} = 0.953$ and $\theta = 1$. The measured values for the adenine nucleotides were then simultated by varying q and θ and solving Eqns. 1–3. From this plot we note that as θ increases, i.e., as the load exceeds the matched value, the minimization of χ^2 tends to overestimate q. Note that the minimum is $\chi^2 = 0$ for all values of θ. Hence, we cannot deduce deviations from conductance matching by inspecting the minimal values of χ^2 obtained in the fit of the experimental data. Therefore, on the basis of χ^2 the choice $\theta = 1$ remains an untestable assumption.

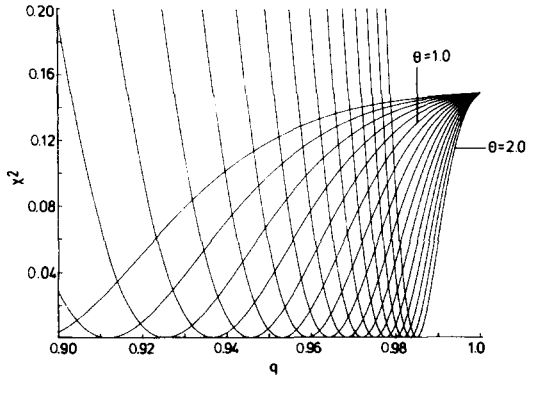

Fig. 2. Dependence of χ^2 on θ. The reference values $[ATP]_{ss}$, $[ADP]_{ss}$ and $[AMP]_{ss}$ were calculated from Eqns. 1–3 for the fixed parameters $X_0 = 184$ kJ, $\theta = 1$, $Z = 2.65$ and $q = 0.953$. The quantity χ^2 (Eqn. 4) was calculated from simulated values $[ATP]_m$, $[ADP]_m$ and $[AMP]_m$, obtained from Eqns. 1–3 by varying q and θ in the range 0.5–2.0 in steps of 0.1.

This situation is different when considering deviations from the adenylate kinase equilibrium, i.e., violation of the steady-state assumption. Fig. 3 depicts χ^2 as a function of q for different values of the mass action ratio $K = [ADP]^2/[ADP][AMP]$. At the equilibrium value of this ratio the minimal χ^2 is again zero but assumes nonzero values for all other values of the parameter K. Simultaneously, the minimum of χ^2 is shifted from the true value of q such that a lowering of the mass action ratio below its equilibrium value tends to overestimate q.

It is instructive to plot the mimimal values of χ^2 as a function of K as done in Fig. 4. Superimposed on this curve are the experimental values of the measured K values and the corresponding minimal χ^2 from the fit of the experimental data (see Results and Discussion). This figure shows that by and large the experimental values exhibit the theoretically predicted behaviour. For this reason we can state that the chosen model, although being very simple, is certainly not completely unrealistic.

This statement could be strengthened if we could prove that the linearity assumptions in our model were valid. However, unfortunately it is impossible to give such a proof. But we can arrive

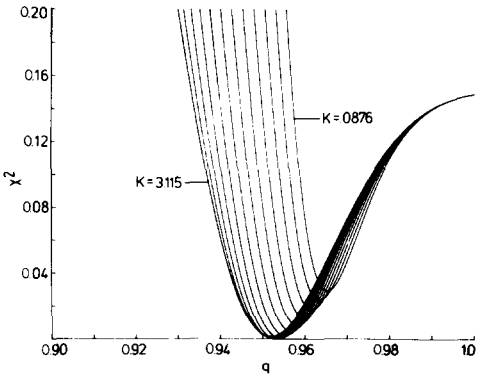

Fig. 3. Dependence on χ^2 on the mass action ratio of the adenylate kinase. The reference values $[ATP]_{ss}$, $[ADP]_{ss}$ and $[AMP]_{ss}$ were calculated as in Fig. 2. The simulated values $[ATP]_m$, $[ADP]_m$ and $[AMP]_m$ as well as χ^2 were again calculated by means of Eqns. 1–4 by varying q and the mass action ratio K (see text). This latter variation was done by changing ΔG^0_{Ak} such that the parameter K took values in the range $0.7 > RT \ln K > -1.5$ in steps of 0.2. Other parameters, as previously mentioned.

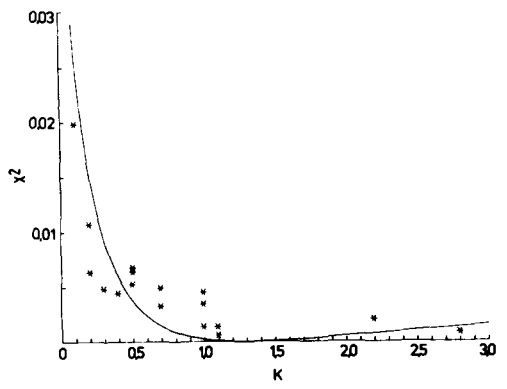

Fig. 4. Dependence of minimal χ^2 on K. This plot is a summary of Fig. 3. The curve was obtained by plotting the minimal values of χ^2 versus the corresponding K. All other parameters, as in previous figures. The starred symbols represent the actual minimal χ^2 values (Table II) and the corresponding K values (Table III) of the experimental data.

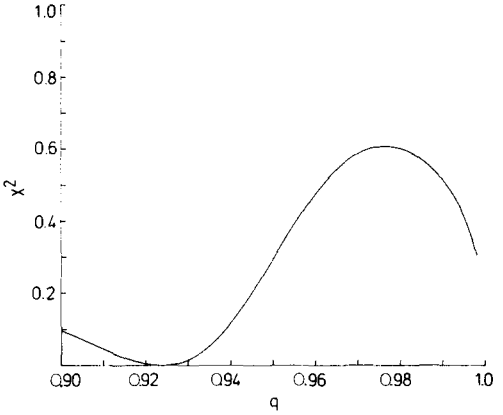

Fig. 5. Dependence of χ^2 on nonlinear relations between flows and forces. The steady-state concentrations of the adeninenucleotides were calculated both for linear and nonlinear phenomenological equations of oxidative phosphorylation as described in the text. The following fixed parameters were used: $X_0 = 184$ kJ, $\theta = 1$, $Z = 2.65$, whereas q was varied within the range indicated in the figure. χ^2 was calculated as the squared distance between corresponding steady-state adeninenucleotide concentrations for the linear and the nonlinear model.

at a plausible argument indicating that deviations from linearity between flows and forces are probably minimal in vivo, as will be explained below.

The problem of linearity versus nonlinearity could be easily solved if the detailed kinetics of oxidative phosphorylation were known. In the absence of such information we have to restrict our analysis to an arbitrary, albeit general model of nonlinear energy converters. Such a model has been proposed by Hill [12] and some of its properties have been worked out in detail. In particular, it was shown that the kinetic constants of a Hill model can be transformed into our dimensionless parameters such as q, Z and θ [13,14]. This allows a direct comparison of linear energy converters with nonlinear ones.

Briefly, we have taken a simple and general nonlinear model of oxidative phosphorylation [12] with an attached load, the latter obeying linear relations between flows and forces. For each value of the dimensionless parameters we have then numerically solved the steady-state equations of this system. From the steady-state concentrations of the adenine nucleotides of the linear and the nonlinear model we have then again calculated χ^2 and plotted this as a function of q in Fig. 5. As is evident from this figure, χ^2 assumes very high values within the range of the values of q determined in this study. A comparison of the χ^2 in Table II with those in Fig. 5 demonstrates that, in general, the linear model allows a much more satisfactory fit of the experimental data than would result with the nonlinear model. This indicates that contaminations of linear relations between flows and forces by possible nonlinearities are probably minimal in vivo.

It must be stressed that there is only one linear model possible whereas there exists an infinity of nonlinear models. Thus, in principle, we should repeat the above calculation of the χ^2 for that infinity of models in order to prove that nonlinearities can indeed be neglected. Since this is obviously impossible our finding has to be interpreted as a plausible argument rather than as a proof against nonlinear contributions in our model.

In summary, we can now state that our simple linear model constitutes a satisfactory approximation of the processes which determine the concentrations of the adenine nucleotides in whole livers, and thus calculate the degree of coupling of oxidative phosphorylation in vivo.

Methods

Hemoglobin-free liver perfusion

Male albino rats (Wistar strain; Thomae Co., Biberach, F.R.G; 180–220 g) received a standard

laboratory diet (Altromin; Lage, F.R.G.) and water ad libitum prior to the isolation of the liver under pentobarbital anaesthesia. Livers were perfused with Krebs-Henseleit bicarbonate buffer [15] (pH 7.4) saturated with O_2/CO_2, 95 : 5 v/v, in a non-recirculating system [16]. Substrates were added as indicated in tables. The stock solution of oleate was prepared as follows: 1 g oleic acid was slowly added to 170 ml albumin solution (20 g/100 ml) under gentle stirring which was continued for a further 2 h at room temperature. In samples of the clear solution, fatty acids were analyzed enzymatically; the oleic acid concentrations were near 20 mM. The calculated ratio of the albumin/oleate complex was 1 : 7. Stock solutions of albumin/oleate complex was 1 : 7. Stock solutions of albumin/oleate or of octanoate were infused into the perfusate immediately prior to its entry into the liver after 50 min of substrate free perfusion via the portal vein. By this procedure the stock solutions were diluted 40-fold. In several experiments the perfusate contained 0.3 mM (2 g/100 ml) of albumin. All perfusion experiments were terminated by freeze-fixation of the livers with a tong precooled in liquid nitrogen [17] after 60 min of perfusion time.

Hemoglobin liver perfusion

Livers from fed rats weighing 230–240 g were infused with 100 ml Krebs-Ringer bicarbonate buffer (pH 7.4) in a recirculating system [18]. The medium contained 4% of bovine serum albumin, 10 g/100 ml hemoglobin which was supplied from washed bovine erythrocytes, 8 mM glucose, 1.5 mM lactate, 0.15 mM pyruvate, 5000 USPE/100 ml heparin and was oxygenated with carbogen. After 40 min of perfusion time the livers were freeze-clamped and freeze-dried.

Extraction of rat liver in vivo

Male rats weighing 200–250 g were either fed on standard laboratory diet or starved for 48 h. Rapid liver sampling was performed by the double-hatchet method [19]. By this method liver samples were taken from anesthetized unrestrained rats and freeze-clamped within 3 s.

Fractionation of tissue in non-aqueous solvents

For determiantion of mitochondrial and cytosolic contents of adenine nucleotides and phosphate the freeze-clamped livers were ground in liquid N_2 and freeze-dried at 0.26 Pa at $-40°C$. About 0.3 g of the freeze-dried powder was sonicated in a mixture of heptane and CCl_4 in 5 s intervals and cooled continuously in heptane/solid CO_2 as described in Ref. 20 and then fractionated on density gradients consisting of heptane/CCl_4 mixtures (density range, 1.28–1.38 kg/l). The procedure was slgithly modified for livers extracted in vivo and hemoglobin-perfused livers [21] to remove erythrocytes. The gradient yielded eight fractions each containing different proportions of mitochondrial and cytosolic protein. Metabolite contents and specific activities of marker enzymes (citrate synthase for mitochondria; phosphoglycerate kinase for cytosol) were measured enzymatically [22] in the fractions and in the unfractionated homogenate.

On the basis of activities of marker enzymes and of adenine nucleotide and phosphate contents in each fraction of the density gradient, mitochondrial and cytosolic metabolite contents were calculated by extrapolation from the fractions to mitochondria and cytosol, respectively [17]. They were converted into concentrations by assuming mitochondrial and cytosolic water contents of 0.8 and 3.8 μl/mg of compartmental protein, respectively [23].

Results and Discussion

In this study we have investigated the regulation of the degree of coupling of oxidative phosphorylation in different systems: (1) perfused rat livers and (2) rat livers in vivo. As explained under Theory we have estimated the degree of coupling in these systems from the measured cytosolic adenine nucleotide concentrations, the lactate/pyruvate ratios and the mean tissue oxygen concentration. Table I summarizes the measured concentrations of adenine nucleotides and phosphate in the cytosol as well as the lactate/pyruvate ratios obtained from perfusate or plasma analyses under different metabolic conditions. Basically there are two major different nutritional states: fed and fasted. In addition, the effects of fatty acids and uncouplers on energy metabolism of perfused livers from fed rats were studied in some

TABLE I

EXTRAMITOCHONDRIAL REDOX RATIOS AND CONCENTRATIONS OF ADENINE NUCLEOTIDES AND PHOSPHATE IN RAT LIVER

Data are means ± S.E.M.; $3 \leqslant 9$.

System	Ratios and concentration (mmol/l)				
	ATP	ADP	AMP	Phosphate	Lactate/pyruvate
Perfused liver					
Fed; no substrates	6.9 ± 0.5	0.67 ± 0.07	0.06 ± 0.01	7.0 ± 0.1 [a]	2.7 ± 0.2
Fed; 8 mM glucose, 2 mM lactate, 0.2 mM pyruvate, hemoglobin [b]	7.2 ± 0.2 [a]	0.90 ± 0.1 [a]	0.04 ± 0.005 [a]	5.1 ± 0.4 [a]	5.0 ± 0 [a]
Fed; 0.5 mM oleate, 2% albumin	4.8 ± 0.5	0.58 ± 0.06	0.18 ± 0.05	6.5 ± 1.0	15 ± 5
Fed; 0.5 mM oleate, 0.5% albumin	4.8 ± 0.4	1.1 ± 0.1	0.23 ± 0.04 [a]	7.1 ± 1.0 [a]	26 ± 4
Fed; 0.5 mM octanoate, 2% albumin	4.6 ± 0.2 [a]	0.43 ± 0.05 [a]	0.15 ± 0.02 [a]	9.9 ± 1.2 [a]	35 ± 7 [a]
Fed; 0.5 mM octanoate	6.0 ± 0.7	1.6 ± 0.3	0.90 ± 0.4	not determined	17 ± 3
Fed; 10 μM FCCP	2.9 ± 0.6	1.9 ± 0.2	1.2 ± 0.4	12.1 ± 1.4	16 ± 3
Fasted; 2 mM dihydroxyacetone	3.3 ± 0.2	1.3 ± 0.03	0.52 ± 0.04	10.0 ± 0.3	6.3 ± 0.1
Fasted; 25 mM glucose	4.5 ± 0.5	0.90 ± 0.12	0.35 ± 0.14	11.6 ± 1.0 [a]	3.9 ± 0.7
Fasted; 25 mM glucose, 1 mM glycerol	3.7 ± 0.2	0.42 ± 0.09	0.42 ± 0.15	7.2 ± 0.6	3.0 ± 0.2
Liver in vivo					
Fed	5.8 ± 0.4	0.78 ± 0.1	0.11 ± 0.06 [a]	4.3 ± 0.3	5 ± 1
Fasted 48 h	4.7 ± 0.2	0.91 ± 0.04	0.08 ± 0.04 [a]	4.3 ± 0.2	18 ± 3

[a] Mean of duplicates.
[b] For details, see Methods.

more detail. Part of these data have already been published and discussed elsewhere in a different context [11,12,24].

The main issue of this paper consists in determining q from the experimental data in order to see whether changes in the metabolic state can affect this thermodynamic parameter. Table II summarizes the results of our estimations of q from the experimental data in Table I as explained in the Theory section. In this table we have listed the estimated degrees of coupling and the associated minimal values of the parameter χ^2 which serves as a figure of merit for the calculations (see Theory). One of the main results of this table is that consistently low values of χ^2 are observed under in vivo conditions. This indicates that the astonishing validity of the extremely simple model assumptions seems to be best fulfilled in the whole animal, rather than under perfusion conditions. One might imagine that an exactly defined condition with specific and controlled metabolic states such as realized in a nonrecirculating perfused liver should result in a much more clearer and more consistent picutre than the same tissue embedded in the very complex environment of a whole organism. However, in contrast to this intuitive picture our data just indicate the contrary to be true. The best values of χ^2 for perfused livers are found exactly in these situations, where a minimum of external manipulations and constraints were applied, such as for example in the case of fed livers perfused without substrates or with physiological substrate concentrations in a closed system. Here, similarly to the situation in the whole animal, the liver presumably has the freedom to adjust its response to the surroundings of a familiar environment instead of being driven into a state where its control mechanisms are notoriously operating at their limits of performance. This importance of the natural environment becomes especially apparent in the case of perfused livers from fasted animals probably because the fasted state of these livers is pushed to such extreme values which seem hardly to be compatible with the survival of the whole animal.

A further inspection of the estimated values of q shows another main result of this study: in the fed animals the degree of coupling is always higher

TABLE II

THE THERMODYNAMIC DEGREE OF COUPLING (q) OF OXIDATIVE PHOSPHORYLATION IN RAT LIVER UNDER DIFFERENT NUTRITIONAL CONDITIONS

Data are means ± S.E.M.; if $n = 2$, mean ± S.D. q has been calculated as described in the Theory section using the single values for metabolite concentrations measured in each experiment. If not measured, the mean values shown in Table I were used.

System	q	χ^2	n
Perfused liver			
Fed; no substrates	0.971 ± 0.004	0.0006	8
Fed; substrates, hemoglobin	0.970 ± 0.001	0.0008	2
Fed; oleate, 2% albumin	0.963 ± 0.009	0.0044	4
Fed; oleate, 0.5% albumin	0.952 ± 0.006	0.0013	4
Fed; octanoate, 2% albumin	0.957 ± 0.007	0.0048	2
Fed; octanoate	0.943 ± 0.008 [a]	0.0067	4
Fed; FCCP	0.925 ± 0.014	0.0045	4
Fed; dihydroxyacetone	0.942 ± 0.001	0.0034	3
Fasted; glucose	0.952 ± 0.002	0.0064	3
Fasted; glucose, glycerol	0..963 ± 0.005	0.0198	4
Liver in vivo			
Fed;	0.970 ± 0.003	0.0013	9
Fasted 48 h;	0.962 ± 0.002	0.0020	9

[a] The value of 9.9 mM was taken for extramitochondrial phosphate assuming that phosphate concentrations were not significantly different with and without albumin. See for example phosphate concentrations for oleate experiments, Table I.

TABLE III

MASS ACTION RATIOS OF THE ADENYLATE KINASE REACTION

For calculation, data of Table I were used.

System	$\frac{[ADP]^2}{[ATP]\,[AMP]}$
Perfused liver	
Fed; no substrates	1.1
Fed; substrates, hemoglobin	2.8
Fed; oleate, 2% albumin	0.4
Fed; oleate, 0.5% albumin	1.1
Fed; octanoate 2% albumin	0.3
Fed; octanoate	0.5
Fed; FCCP	1.0
Fasted; dihydroxyacetone	1.0
Fasted; glucose	0.5
Fasted; glucose, glycerol	0.1
Liver in vivo	
Fed	1.0
Fasted 48 h	2.2

than in the fasted animals. In the fasted state, however, the q values show a greater variation than in the fed state. For example on perfusing with glucose plus glycerol the degree of coupling seems to be rather high being between the values found for the fed and fasted state. Here we need to have a closer look at the assumptions and limitations underlying our method for the estimation of q. Table III shows that in this case the actual mass action ratio of the adenylate kinase reaction is farthest from the equilibrium value. In other words, there is a net driving force for the adenylate kinase reaction leading to a net flow of AMP. Hence, contrary to our initial assumption, the phosphorylating system is not in a true steady state although it may be quite close to it. No wonder that under these conditions a marked deviation of χ^2 from zero can be observed. This shows quite clearly that in principle our simple model can no longer be applied to this particular situation. What can be salvaged from this dilemma? Here Fig. 4 is of help. As pointed out in the Theory section this plot shows the theoretically expected dependence of the minimal value of χ^2 on the disequilibrium of the adenylate kinase based on our model. A plot of the actually measured minimal χ^2 vs. the actually observed mass action ratios obtained from the experimental data demonstrates, that the expected dependence is fulfilled quite satisfactorily. Hence, our model can also be applied to situations not too far from steady state showing that we can, with some confidence, examine the deviations of the calculated from the true values of q as a function of the disequilibrium of the adenylate kinase. To examplify this point we note from Fig. 3 by using Tables II and III that for this extreme case of fasted livers perfused with glucose plus glycerol where we calculated $q = 0.963$ this value is most probably overestimated by about 0.01. This apparent disequilibrium of the adenylate kinase seems to be induced by the high rate of activation of glucose for glycolysis and of fatty acids for lipid synthesis (due to the addition of glucose and glycerol) thus disturbing cytosolic adenine nucleotides. In addition to this obvious exception there are other smaller exceptions to the general rule

which we leave to the reader to explain according to the same reasoning as explained above based on Fig. 3 and Tables II and III.

On the other hand, such an explanation does not hold for the rather high q observed in the fasted state in rat liver in vivo, where deviations of the calculated q from the 'true one' cannot be explained on the basis of a disequilibrium of the adenylate kinase reaction. In this case, it should be pointed out again that the situation of fasting in vivo for 48 h is not comparable to the one with livers extracted from the animals and depleted of substrates during isolation and initial substrate-free perfusion.

The different values for the degree of coupling in the fed and fasted state raise two main questions: (1) how manages the cell to change q and (2) for what purpose? The first question leads us to the experiments where livers from fed rats were supplied with fatty acids such as oleate and octanoate. Interestingly, the degree of coupling is decreased to values similar to those of the fasted state, i.e., about 0.95. This effect is concentration dependent. Thus when decreasing the amount of free fatty acids by adding albumin, the values found for q are about 0.96. Decrease of coupling of oxidative phosphorylation by free fatty acids is well known from experiments with isolated mitochondria [3–5] and perfused liver [24, although the effect is clearly not comparable to the effect of a classical uncoupler such as FCCP (Table II).

At this point it should be noted that also in the fasted state fatty acids are increased in liver in comparison to the fed state; this is reflected by elevated plasma free fatty-acid concentrations and tissue contents of long-chain acyl-CoA [6,25]. Observations consistent with our findings were made by Klug et al. [26], who found that in liver mitochondria from rats trained exhaustively (a situation metabolically similar to fasting), and/or fasting, the ADP/O ratio was reduced by 50%. The authors attributed this effect to the influence of free fatty acids, since albumin could reestablish the ADP/O ratio.

It is, therefore, conceivable that an additional function of the fatty acids, besides their role as metabolic fuels, is the regulation of q. What, however, is the advantage of having a lower q in the fasted state? Intuitively, one would expect that especially under this condition where substrate is lacking it would be of greatest advantage to get as much ATP from oxidation of substrates as possible. This apparently logical consideration, however, does not care about optimal efficiency of energy conversion in different functional states.

As stated in the introduction, the economic degrees of coupling of oxidative phosphorylation for a maximal power and a maximal flow situation are $q_p^{ec} = 0.972$ and $q_f^{ec} = 0.953$, respectively. Our values of q estimated for the fed and the fasted state in vitro and in vivo are strikingly close to these theoretical values. Thus, operating at lower q in the fasted state enables the liver to synthesize glucose at high rates despite a lowered cytosolic phosphate potential X_p, which apparently is still sufficiently high to drive gluconeogenesis [11]. On the other hand, a higher cytosolic X_p (as a consequence of an increasing q) is clearly more favourable if very high rates of biosynthetic processes are not necessary as under normal fed conditions. The striking consistency of theoretically postulated degrees of coupling at optimal efficiency with the values found in the fed and fasted state indicates that the liver cell realizes conductance matching and validates the assumption of $\theta = 1$ made in the Theory section. This regulation of q by fatty acids in order to achieve optimal efficiency and economy in the cell would be an impressive example of the evolutionary design of energy conversion.

Finally, one should be aware of the fact that the nonphysiological substrate octanoate does not only influence the degree of coupling, but the whole energy converting system itself. This can be surmised from the very high χ^2 observed at high octanoate, which cannot only be explained by a deviation from the adenylate kinase equilibrium (see Fig. 4 and Table III). This situation is similar to the case of infusion of FCCP where adenylate kinase is in nearly perfect equillibrium, but χ^2 is rather high. One may speculate that due to the very high rates of oxygen consumption under these conditions (165% of control with octanoate and 200% of control with FCCP) oxygen supply in hemoglobin-free perfused liver is at its limits, and therefore linearity between flows and forces is compromised. Further, since both of these substances are nonphysiological, this may be an ad-

ditional reason for a possible deviation from linearity far from equilibrium [14].

One may ask the question of how fatty acids regulate the degree of coupling of oxidative phosphorylation. According to the conventional picture of the action of uncouplers one might surmise that fatty acids act as protonophores, which partially collapse the $\Delta\mu H^+$ across the inner mitochondrial membrane. This means that fatty acids would introduce a passive leak into a membrane conductance. Recently, a different possibility for uncoupling has been published: slip. Slippage means not a passive leak in a membrane, but rather a direct interference of a substance with redox and/or ATPase proton pumps [27]. Since slippage is thus linked directly to the coupling mechanism of oxidative phosphorylation, it is therefore susceptible to inhibition of the electron flux through the respiratory chain, in contrast to passive leaks. Hence, it is possible to decide whether a lower q is the result of leaks or slips by titrating isolated mitochondria with respiratory inhibitors and examining the dependence of net oxygen consumption on the steady-state phosphate potential. A recent study has shown that oleate and palmitate lower q almost exclusively by a slip mechanism (Stucki, J.W., unpublished data). This specific mechanism of fine tuning of q by fatty acids would allow the cell to follow closely the changing energy requirements imposed by the outside world.

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