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KINETICS OF GLYCEROL UPTAKE BY THE PERFUSED RAT LIVER

MEMBRANE TRANSPORT, PHOSPHORYLATION AND EFFECT ON NAD REDOX LEVEL

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SUMMARY

The kinetics of glycerol uptake by the perfused rat liver were determined according to a model which includes membrane transport, intracellular phosphorylation and competitive inhibition of glycerol phosphorylation by L-glycerol 3-phosphate. The membrane transport obeys first-order kinetics at concentrations below 10 mM in the affluent medium. The K_m of the glycerol phosphorylation was 10 μM and the K_i of the L-glycerol 3-phosphate inhibition was 50 μM. The maximum activity (V) was 3.70 μmoles/min per g liver wet wt. These results are similar to in vitro kinetics of the glycerol kinase, except that K_i was found to be somewhat lower in the intact organ. At low glycerol concentrations, a steep concentration gradient exists across the liver cell membrane.

The increase in the lactate to pyruvate concentration ratio during glycerol metabolism is related to the actual concentration of L-glycerol 3-phosphate, not to the rate of glycerol uptake.

INTRODUCTION

Glycerol is used by the liver as a precursor for gluconeogenesis or triglyceride synthesis or as a substrate for oxidation. The rate of glycerol uptake is regulated by several factors including the concentration of glycerol in the blood [1, 2], the nutritional [3] and the thyroid state [4] or the simultaneous metabolism of other substrates such as sorbitol or ethanol [5, 6]. The first step of glycerol metabolism is an irreversible phosphorylation by glycerokinase, the activity of which is regulated by AMP and L-glycerol 3-phosphate in vitro [7, 8, 9]. However, the regulatory properties of the enzyme as measured in vitro do not seem to conform very well to in vivo observations. The kinetics of the glycerol elimination by the intact organ were, therefore, analyzed in terms of a model system including membrane transport, intracellular phosphoryla-

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tion and feedback inhibition by the product of phosphorylation, L-glycerol 3-phosphate. Further, the time–concentration dependence of the lactate to pyruvate concentration ratio, adenine nucleotides and inorganic phosphate were investigated.

MATERIALS AND METHODS

Analytical grade reagents were employed when available. Glycerol was supplied by Merck, Darmstadt. Enzymes and coenzymes were from Boehringer and Soehne, Mannheim, Germany. Animals were female Wistar rats, weighing 170 g. Glycerol, L-glycerol 3-phosphate [10], glucose [11], lactate [12], pyruvate [13], ATP [14], ADP and AMP [15] were determined enzymatically. The rat liver perfusion technique has been described in detail previously [16]. A non-recirculating perfusion system was employed using Krebs–Ringer bicarbonate buffer and washed bovine erythrocytes at a hematocrit of 28 %. The lactate concentration in the affluent medium was about 1 mM. The gas phase was air, to which 5 % carbon dioxide was added. The extracellular volume was determined to be 179 μl/g liver at a perfusion pressure between 5 and 10 cm of water. The elimination rate of glycerol was observed at different concentrations of L-glycerol 3-phosphate, by perfusing the liver with different concentrations of glycerol, which were abruptly changed. All rates used for calculations were measured simultaneously with the performance of a biopsy after 8 min of perfusion with a constant glycerol concentration in the affluent medium.

Whenever biopsies were performed during the perfusion the flow was reduced proportionally to the weight reduction. This could be done because the weight of the lower left lobe is very constant and amounts to 30 % of the total liver weight. The flow was 0.75 ml/g liver wet wt. Rates are given as μmole/min per g liver wet wt. Values are given $\pm$S.E.

MODEL FOR GLYCEROL ELIMINATION BY PERFUSED RAT LIVER

The basic assumption is that the rate of glycerol uptake by the liver is governed by two processes, a carrier-mediated equilibrated membrane transport followed by an irreversible phosphorylation by an enzyme (glycerokinase), which is subjected to competitive inhibition by the product (L-glycerol 3-phosphate). The liver is described by a simplified model, in which all geometrical quantities and concentrations of the relevant metabolites depend only on the distance from the place of entrance of the affluent medium. It is then possible to relate the concentration of glycerol in the affluent and the effluent media and the biopsy concentration of glycerol and L-glycerol 3-phosphate to the liver geometry and to the kinetic constants of the processes assumed to be involved.

A similar method applied to the elimination of fructose has been described previously [17]. The glycerol concentration is assumed to be identical in the sinusoids, in the interstitial space [18], and in the erythrocyte water phase. L-glycerol 3-phosphate is assumed to be present only in the hepatocytes. Both glycerol and L-glycerol 3-phosphate are assumed to be equally distributed within each hepatocyte. No correction for intracellular dry matter has been performed.

The glycerokinase reaction

Glycerol + ATP → L-glycerol 3-phosphate + ADP

This reaction phosphorylates an amount of glycerol per time unit equal to:

$$\frac{V_2 S_I}{S_I + K_2\left(1 + \frac{i}{K_i}\right)} m\Delta x \tag{1}$$

between x and $x+\Delta x$. Since the K_m of glycerokinase with ATP is low [9], saturating concentrations can be assumed to be always present. During the steady state, this amount is equal to the net transport of glycerol across the cell membrane between the same limits of x:

$$\left(\frac{V_1 S}{S + K_1} - \frac{V_1 S_I}{S_I + K_1}\right) m\Delta x \tag{2}$$

This expression in turn is equal to the amount of glycerol that has disappeared from the sinusoid between x and $x+\Delta x$:

$$-F(1-\alpha)\frac{dS}{dx}\Delta x \tag{3}$$

The biopsy concentration of glycerol is given by

$$S_B = \frac{1}{\rho}[\bar{S}_I(1-\gamma-\delta) + \bar{S}(\gamma + (1-\alpha)\delta)] \tag{4}$$

where

$$\bar{S}_I = \int_0^1 S_I dx,\ \bar{S} = \int_0^1 S dx \tag{5}$$

If S_I is eliminated from (1), (2) and (3) by equating the expressions then

$$-\frac{y(v+1) + c + \sqrt{ay^2 + by + c^2}}{y} dy = I \cdot dx \tag{6}$$

where:

$y = S/K_1$

$a = (v-1)^2 + 4vk;\ b = 2(v-1)(v+k) + 4k(v+1);\ c = v+k$

$$v = V_2/V_1; \qquad k = K_2\left(1 + \frac{i}{K_i}\right)/K_1; \qquad I = \frac{vV_1}{K_1}\frac{2m}{F(1-\alpha)}$$

Equation (6) is integrated to give

$$\int_{S_E/K_i}^{S_A/K_1} \frac{y(v+1) + c + \sqrt{ay^2 + by + c^2}}{y} dy = I \tag{7}$$

If it is assumed that it is independent of x, then S_E may be calculated from this equation if S_A, i, and the kinetic constants are known (cf. ref. 17). The value of i is taken to be the intracellular mean L-glycerol 3-phosphate concentration i.e. the biopsy concentration multiplied by

$$\frac{\rho}{1-\gamma-\delta}.$$

Further, the biopsy concentration of glycerol may be calculated from equations (4) and (5), when S_E has been determined. When a series of corresponding values of S_A, S_B, S_E and i has been observed it is possible to determine the kinetic constants K_1, V_1, K_2, V_2, and K_i by minimizing the sum of the deviations of the observed from the calculated values of S_E and S_B for each S_A (see Table II):

$$\sum_{S_A} \left\{ \left(\frac{S_E(\text{obsd}) - S_E(\text{cald})}{S_E(\text{cald})} \right)^2 + \beta \sum_{S_A} [S_B(\text{obsd}) - S_B(\text{cald})]^2 \right\}$$

where β is introduced in order to obtain identical dimensions of the two terms. The magnitude of this constant is chosen such that the terms are weighted equally (for details, see ref. 17). For computation a Univac 1110 was employed.

TABLE I

LIST OF DEFINITIONS USED IN THE CALCULATIONS

Symbol	Unit	Definition
x	—	Distance from the portal end of the sinusoid divided by the sinusoid length
V_1	μmole/min per g	Maximum velocity of the membrane transport of glycerol
K_1	mM	Michaelis constant of the membrane transport of glycerol
V_2	μmole/min per g	Maximum velocity of the glycerokinase reaction
K_2	mM	Michaelis constant of the glycerokinase reaction with glycerol
K_i	mM	Inhibitor constant of the glycerokinase reaction with L-glycerol 3-phosphate
m	g	Liver weight
S	mM	Concentration of glycerol in the extracellular water phase
S_I	mM	Concentration of glycerol in the intracellular space
i	mM	Concentration of L-glycerol 3-phosphate in the intracellular space
F	ml/min	Flow of total perfusate
α	—	Hematocrit (L-glycerol concentration in erythrocytes/S)
ρ	g/ml	Specific gravity of liver
γ	—	Interstitial volume
δ	—	Vascular volume/entire liver volume
$\bar{S}$	mM	Mean value of S
$\bar{S}_I$	mM	Mean value of S_I
S_B	μmoles/g	Concentration of glycerol in the biopsy
S_A	mM	Value of S at $x = 0$
S_E	mM	Value of S at $x = 1$

RESULTS AND DISCUSSION

The kinetic theory employed is a steady state theory. Regarding the rate of uptake of glycerol, a steady state was attained after 8 min of perfusion, whereas the steady state was not attained in the cytosolic NAD redox level at high glycerol concentrations (Fig. 3). However, since no accumulation of glycerol takes place in the liver (the biopsy concentrations are small compared to the rate of glycerol uptake) and since the glycerol kinase inhibition by L-glycerol 3-phosphate must be considered immediate, the approximation to steady state must be considered acceptable.

Kinetics of glycerol uptake

The relation between glycerol concentration and rates of glycerol uptake by perfused rat liver is given in Fig. 1. The highest rate of glycerol uptake is about 2.3 μmoles/min per g liver which corresponds well to the activity of glycerokinase [20]. Saturation is attained at a concentration of glycerol above 5 mM in the affluent medium corresponding to about 2 mM in the effluent medium. This is not in accordance with previous studies using rat liver slices [3], perfused rat liver [19], rat hepatocytes [5], cats or humans beings in which the saturating concentration was found to be about 1 mM. However, the K_m value of glycerokinase with glycerol is 100 times lower, about 10 μM [9]. This suggests that the rate of glycerol uptake by the liver cannot be determined solely by the kinetics of the first glycerol-metabolizing intracellular step.

The figures on which calculations of the kinetic constants were based are given in Table II. In Table III the calculated kinetic constants of glycerol uptake are given together with the relevant kinetic parameters obtained from in vitro studies. The K_m value for membrane transport given in Table III is not a definite value since it is impossible to distinguish between this and higher values, on the basis of the affluent glycerol concentrations employed in the present investigation. However, it can be stated that at physiological glycerol concentrations, the membrane transport obeys first-order kinetics.

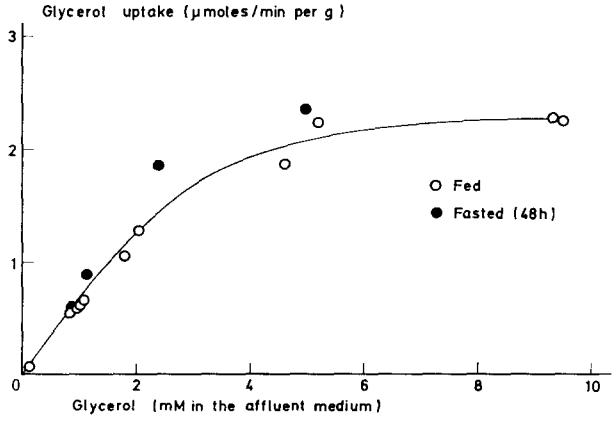


Fig. 1. Glycerol uptake in the perfused liver. Livers were perfused for 8 min at a constant concentration of glycerol in the affluent medium (abscissa). ○, fed rats; ●, rats fasted for 48 h.

TABLE II

OBSERVED CONCENTRATIONS

Measured concentrations on which the calculations were based and the concentrations of ATP and AMP. Different concentrations of L-glycerol 3-phosphate were obtained by preceding perfusion with glycerol at higher concentrations. Therefore, the concentration of ATP cannot be related to the glycerol concentration given in the first column. In control perfusion without glycerol added, the biopsy concentrations were: Glycerol: 0.05±0.01, L-glycerol 3-phosphate: 0.29±0.05, ATP: 2.60± 0.06, AMP: 0.40±0.01 (mean ±S.E., $n = 4$).

Affluent medium Glycerol (mM) S_A	Effluent medium Glycerol (mM) S_E	Biopsies				Deviation	
		Glycerol (μmole/g) S_B	Glycerol 3-*P* (μmole/g) i	ATP (μmole/g)	AMP (μmole/g)	Observed (mM) S_E	Calculated (μmole/g) S_B
1.14	0.18	0.04	0.58	1.75	0.65	0. 00	−0.06
1.37	0.25	0.13	1.20	2.00	0.43	−0.02	−0.02
1.45	0.21	0.14	2.06	1.78	0.23	0.03	−0.02
1.34	0.34	0.31	5.99	2.33	0.35	0.02	0.05
2.47	0.46	0.41	1.97	2.60	0.46	−0.08	0.08
2.81	0.54	0.00	2.02	—	—	−0.13	—
7.12	2.58	1.63	4.64	2.58	0.27	−0.09	−0.27
6.31	2.60	1.57	7.14	1.78	0.44	0.00	−0.33
12.51	8.32	5.17	7.13	1.07	0.64	0.80	0.39
13.01	8.60	4.72	8.09	2.28	0.33	0.50	−0.40

TABLE III

KINETIC CONSTANTS OF GLYCEROL UPTAKE BY PERFUSED RAT LIVER

The kinetic parameters were calculated according to the model outlined above using the experimental observations given in Table II. K_1 and V_1 are minimum values because glycerol concentrations which were able to saturate the membrane transport system were not attained. References are given in parenthesis.

	K_1 (mM)	V_1 (μmole/min per g)	K_2 (mM)	V_2 (μmole/min per g)	K_i (mM)
Calculated from observations on perfused rat liver	15	15	0.01	3.66	0.05
Calculated from observations on in vitro preparations	—	—	0.01 [9]	2.08 [20]	0.39 [8] 0.58 [7]

The calculated K_m for the intracellular phosphorylation of glycerol is of the same magnitude as the K_m of the glycerokinase with glycerol measured using a partially purified enzyme preparation [9]. This supports the tenability of the model for glycerol elimination by the liver, and indicates that a steep concentration gradient of glycerol exists across the cell membrane at low glycerol concentrations in the affluent medium (<5 mM). Inhibition of the glycerokinase reaction by L-glycerol 3-phosphate has been shown to occur only in media not containing inorganic phosphate or sulfate [8]. Therefore, it should be expected that this kind of inhibition was of no

significance in the intact organ, in which inorganic phosphate is present at concentrations of about 4 μmoles/g [16]. However, calculations of the kinetics of glycerol uptake showed that a much better fit of observed to calculated values of S_E and S_B was obtained when the inhibition of the phosphorylation step by L-glycerol 3-phosphate was introduced (Table II). This inhibition also explained the fact that the intracellular concentration of glycerol was not only dependent on the extracellular concentration of glycerol, but increased by a factor of 10 with increasing concentrations of L-glycerol 3-phosphate (Table II). The inhibitor constant was calculated to be 50 μM which is somewhat lower than the value obtained in vitro (Table III). This may be due to differences in the ionic milieu in the cell and in vitro, or to compartmentation of L-glycerol 3-phosphate in the hepatocyte. However, the fact that the calculations are based on the average inhibitor concentration may also introduce some error. Inhibition of glycerol phosphorylation by AMP was not taken into consideration in the model because the kinetics of this kind of inhibition could not be formally described [9]. However, the concentration of AMP was measured in the experiments, and no correlation between the AMP concentration and the degree of inhibition could be established (Table II).

One consequence of the kinetics of glycerol uptake by the intact liver, as described here, is that at physiological glycerol concentrations, i.e. < 1 mM, the rate of glycerol uptake is virtually independent of the actual concentration of L-glycerol 3-phosphate. The mechanism is, that an increase in the inhibition of glycerol phosphorylation is counterpoised by an increase in the concentration of glycerol in the hepatocyte. Since the intracellular glycerol concentration increases in a concentration range, in which the activity of the enzyme is dependent on the substrate concentration.

TABLE IV

FLUXES OF GLYCEROL ACROSS THE LIVER CELL MEMBRANE

The figures demonstrate that at increasing concentrations of L-glycerol 3-phosphate the efflux of glycerol is increased greatly (e.g. at 1 mM glycerol in the affluent medium). Since the absolute value of the efflux is small compared to the influx, the increase only causes insignificant changes in the net rate of glycerol uptake. An inhibition of the glycerokinase reaction is counteracted by an increasing mean concentration of glycerol both in the extracellular and in the intracellular phase.

Observed values	Calculated values			
Rate of glycerol uptake (μmole/min per g)	Net rate of glycerol uptake (μmole/min per g)	Glycerol influx (μmole/min per g)	Glycerol efflux (μmole/min per g)	Mean extracellular glycerol (mM) $\bar{S}$
0.51	0.49	0.51	0.02	0.53
0.63	0.61	0.66	0.05	0.69
0.54	0.52	0.60	0.08	0.62
0.51	0.50	0.70	0.20	0.72
1.08	1.03	1.20	0.17	1.30
1.24	1.19	1.40	0.21	1.53
2.25	2.22	3.62	1.40	4.71
1.92	1.90	3.38	1.47	4.30
2.72	2.72	6.07	3.35	9.95
2.69	2.69	6.27	3.57	10.50

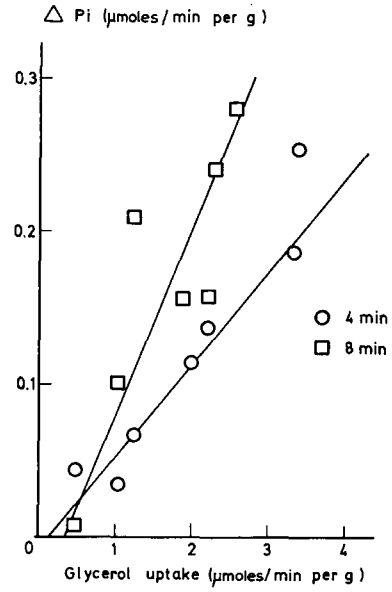
The net rate of glycerol uptake also increases. At higher concentrations of glycerol the inhibition by L-glycerol 3-phosphate will cause a decrease in the rate of glycerol uptake, although an increase in intracellular glycerol will tend to diminish the competitive inhibition by L-glycerol 3-phosphate.

Metabolic consequences of glycerol uptake

As has been shown above, the entry of glycerol into the liver is regulated by a sequence of processes, the membrane transport and the intracellular phosphorylation. For the evaluation of the metabolic effects of glycerol on liver, it is important to consider the effect of glycerol phosphorylation and the effect of L-glycerol 3-phosphate metabolism separately.

The phosphorylation of glycerol causes a decrease in the hepatic content of ATP [19, 21]. In experiments with fructose, the decrease in ATP concentration was directly correlated to the rate of fructose uptake [17]. This was not the case in experiments with glycerol (Table II). In experiments with glycerol, steady state conditions were not attained, and therefore the concentration of ATP may have been influenced by differences in the cytosolic NAD redox level (via glyceraldehyde-3-phosphate dehydrogenase equilibrium); or by preceding glycerol uptake at a higher rate.

The accumulation of glycerol 3-phosphate causes a decrease in the hepatic concentration of inorganic phosphate [19, 21], and gives rise to an increased rate of phosphate uptake (Fig. 2).



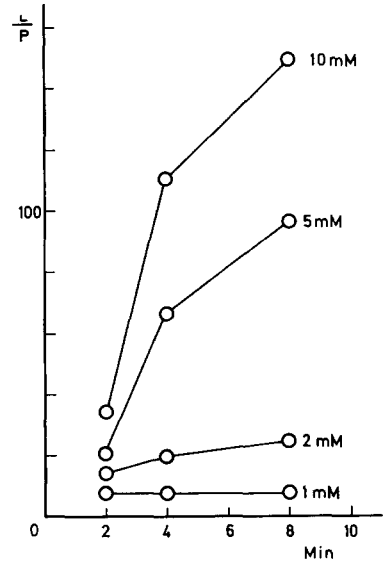


Fig. 2. Changes in phosphate uptake induced by glycerol in perfused liver. The rate of phosphate uptake was calculated as the difference between the rate of uptake before and 4 and 8 min after initiation of perfusion with glycerol in livers from fed rats.

Fig. 3. Effect of glycerol on the lactate to pyruvate concentration ratio in the effluent medium in perfused livers. The lactate to pyruvate concentration ratio increases immediately after the addition of glycerol. The rate of change and the magnitude of the change depends on the time elapsed since the addition of glycerol and on the concentration given.

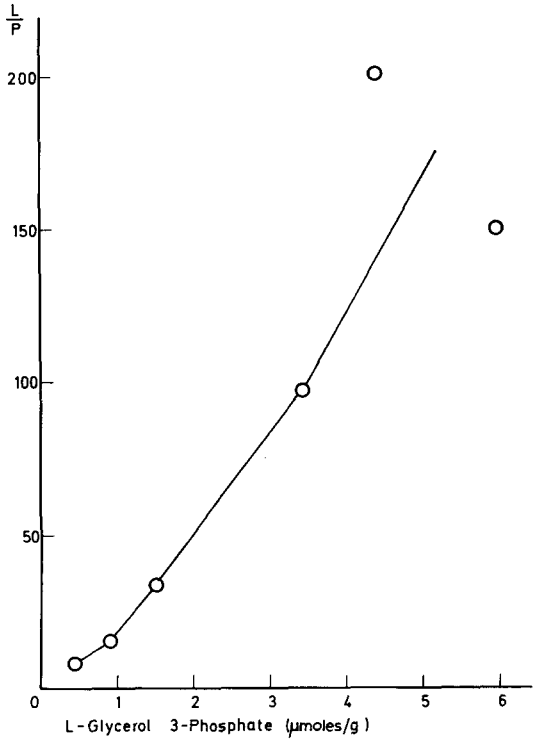


Fig. 4. Effect of L-glycerol 3-phosphate on lactate to pyruvate concentration ratio in perfused livers. The livers were initially perfused with different concentrations of glycerol in order to attain different concentrations of L-glycerol 3-phosphate in the liver. Then the concentration of glycerol in the affluent medium was changed to 1 mM in all experiments, and the lactate to pyruvate concentration ratio and the biopsy concentration of L-glycerol 3-phosphate was determined after 8 min further perfusion. 1 mM glycerol in the affluent medium caused no change in the lactate to pyruvate concentration ratio in itself (Fig. 3), whereas the ratio was correlated to the concentration of L-glycerol 3-phosphate.

The first step in the further metabolism of L-glycerol 3-phosphate is an oxidation to dihydroxyacetone phosphate either in the cytosol or intramitochondrially. The introduction of a reduced substrate as glycerol has repeatedly been shown to change the equilibrium of the cytosolic NAD redox couple, lactate and pyruvate, in the direction of the reduced compound [19, 22]. The relation between the lactate to pyruvate concentration ratio in relation to time and glycerol concentration is shown in Fig. 3. The actual lactate to pyruvate concentration ratio in hepatic effluent blood is, however, related to the rate of glycerol uptake via the coincident concentration of L-glycerol 3-phosphate (Fig. 4). Since the concentration of L-glycerol 3-phosphate determines the rate of cytosolic NADH formation at the glycerolphosphate dehydrogenase step, the effect of glycerol uptake on cytosolic redox pairs must logically be correlated to the actual concentration of L-glycerol 3-phosphate and not to the rate of glycerol uptake. However, the effect on the lactate to pyruvate ratio may also, in part, be secondary to changes in the mitochondrial NAD redox level.

Recent studies with rat hepatocytes have shown that the rate of glycerol, as well as sorbitol uptake, is ultimately determined by the actual rate of hydrogen transfer

from cytosol to mitochondria [23]. In this respect the L-glycerol 3-phosphate shuttle seems to play a major role, and the rate of metabolism of L-glycerol 3-phosphate is thus determining for the rate of glycerol phosphorylation. One likely mechanism for such a feedback control on glycerol phosphorylation is inhibition by L-glycerol 3-phosphate, which according to the present studies seems to play a role in the intact organ.

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