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Disequilibrium in the Triose Phosphate Isomerase System in Rat Liver

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1. The equilibrium constant at 38° and I 0·25 of the triose phosphate isomerase reaction was found to be 22·0 and that of the aldolase reaction, $0{\cdot}99 \times 10^{-4}$M. The [dihydroxyacetone phosphate]/[glyceraldehyde phosphate] ratio was found to be 9·3 in rat liver. The causes of the apparent deviation of the triose phosphate isomerase system from equilibrium *in vivo* have been investigated. 2. The equilibria of the triose phosphate isomerase and aldolase reactions were studied with relatively large concentrations of crystalline enzymes and small concentrations of substrates, approximating to those found in rat liver and muscle. There was significant binding of fructose diphosphate by aldolase under these conditions. There was no evidence that binding of glyceraldehyde phosphate by either enzyme affected the equilibria. 3. The deviation from equilibrium of the triose phosphate isomerase system in rat liver can be accounted for by the low activity of the enzyme, in relation to the flux, at low physiological concentrations of glyceraldehyde phosphate (about 3μM). It has been calculated that a flux of 1·8μmoles/min./g. wet weight of liver would be expected to cause the measured degree of disequilibrium found *in vivo*. 4. The conclusion that the triose phosphate isomerase is not at equilibrium is in accordance with the situation postulated by Rose, Kellermeyer, Stjernholm & Wood (1962) on the basis of isotope-distribution data. 5. The triose phosphate isomerase system is closer to equilibrium in resting muscle probably because of a very low flux and a high enzyme concentration. 6. The aldolase system deviated from equilibrium in rat liver by a factor of about 10 and by a much greater factor in resting muscle. 7. The measurement of total dihydroxyacetone phosphate and glyceraldehyde phosphate content indicates the concentrations of the free metabolites in the tissue. This may not hold for fructose diphosphate, a significant proportion of which may be bound to aldolase.

Information on the concentrations of the free triose phosphates in tissues is of special interest because it can, under certain conditions, be used for calculating a relationship between the redox state of the NAD couple and phosphorylation state of the adenine nucleotide system in the cytoplasm (Krebs & Veech, 1969). Such calculations are justified provided that the reactants of the following processes are at near-equilibrium concentrations in the cytoplasm:

$$\text{Lactate} + \text{NAD}^+ \rightleftharpoons \text{Pyruvate} + \text{NADH} \quad (1)$$

$$\text{Glyceraldehyde 3-phosphate}^* + \text{NAD}^+ + \text{P}_i \rightleftharpoons \text{1,3-Diphosphoglycerate} + \text{NADH} \quad (2)$$

$$\text{1,3-Diphosphoglycerate} + \text{ADP} \rightleftharpoons \text{3-Phosphoglycerate} + \text{ATP} \quad (3)$$

At equilibrium, from reactions (2) and (3):*

$$\frac{[\text{NADH}]}{[\text{NAD}^+]} = K_{\text{GAP}} \cdot K_{\text{3PG}} \cdot \frac{[\text{GAP}]}{[\text{3PG}]} \cdot \frac{[\text{ADP}][\text{P}_i]}{[\text{ATP}]} \quad (4)$$

* Abbreviations (for kinetic expressions): 3PG, 3-phosphoglycerate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; FDP, fructose 1,6-diphosphate; K_{TPI}, equilibrium constant of the triose phosphate isomerase reaction; K_{aldolase}, equilibrium constant of the fructose 1,6-diphosphate adolase reaction; K_{LDH}, equilibrium constant of the lactate dehydrogenase reaction; K_{GAP}, equilibrium constant of the glyceraldehyde 3-phosphate dehydrogenase reaction; K_{3PG}, equilibrium constant of the 3-phosphoglycerate kinase reaction.

The value for [NADH]/[NAD$^+$] (where these are the free nicotinamide nucleotides) can be calculated from the equilibrium expression for reaction (1), and substitution in eqn. (4) gives:

$$\frac{[\text{lactate}]}{[\text{pyruvate}]} = K \cdot \frac{[\text{GAP}]}{[\text{3PG}]} \cdot \frac{[\text{ADP}][\text{P}_i]}{[\text{ATP}]} \qquad (5)$$

where

$$K = K_{\text{GAP}} \cdot K_{\text{3PG}} / K_{\text{LDH}}$$

If the equilibrium constants are measured in the usual way with catalytic concentrations of the enzymes, the concentrations of metabolites in these equations refer to the free, as opposed to protein-bound, substances. It is highly probable that lactate, pyruvate, P_i, ADP, ATP and 3-phosphoglycerate are not significantly bound because the concentrations of the specific proteins (the enzymes) that bind these substances are very much lower than those of the metabolites. It is, however, uncertain whether glyceraldehyde 3-phosphate, as measured in animal tissues, represents free glyceraldehyde 3-phosphate because the concentrations of total glyceraldehyde 3-phosphate (about 3μM) are of the same order of magnitude as the concentrations of the three enzymes (aldolase, triose phosphate isomerase and glyceraldehyde phosphate dehydrogenase) that can bind glyceraldehyde 3-phosphate. More important still, despite the high activity of triose phosphate isomerase in tissues indicated by assays, the relative concentrations of total dihydroxyacetone phosphate and total glyceraldehyde 3-phosphate found in tissues are not those expected if the triose phosphate isomerase reaction is at equilibrium (Hess, 1963; Williamson, Kreisberg & Felts, 1966; Minakami & Yoshikawa, 1966). This deviation could be due either to preferential binding of glyceraldehyde 3-phosphate or to true non-equilibrium. That the triose phosphate isomerase system is not at equilibrium *in vivo* under some conditions has in fact been postulated by Ljungdahl, Wood, Racker & Couri (1961) and Rose *et al.* (1962) on the basis of isotope distribution data in glucose synthesized from glycerol or lactate. There is not necessarily a contradiction between this postulate and the high tissue content of triose phosphate isomerase because the concentrations of substrates in the tissues are much lower than their K_m values.

The work reported in the present paper indicates that binding of glyceraldehyde 3-phosphate is not responsible for major deviations *in vivo* from the equilibrium value in the triose phosphate isomerase system and that true non-equilibrium is the most satisfactory explanation of the observed deviations. The net flux between glyceraldehyde 3-phosphate and dihydroxyacetone phosphate calculated from the observed disequilibrium is of the same order as that expected from the rate of glycolysis in the liver of well-fed rats or the rate of gluconeogenesis from lactate in the liver of starved rats.

MATERIALS AND METHODS

Animals. Male rats of the Wistar strain, weighing 120–160g., were used.

Reagents. Rabbit muscle aldolase and triose phosphate isomerase, glycerol 1-phosphate dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, fructose 1,6-diphosphate, dihydroxyacetone phosphate dimethylketal, glyceraldehyde phosphate diethylacetal, NAD$^+$ and NADH were obtained from Boehringer Corp. (London) Ltd., London W.5. Dihydroxyacetone phosphate and glyceraldehyde 3-phosphate were prepared by hydrolysis of the ketal and acetal derivatives at pH 1·5–2·0, at 40°, for 6hr. and 48hr. respectively.

Treatment of liver and muscle. Rat liver and muscle extracts were prepared by the freeze-clamping technique as described by Williamson, Lund & Krebs (1967) and Veech, Eggleston & Krebs (1969). The animals from which samples of muscle were obtained were under ether anaesthesia. This made it possible to expose the posterior femoral muscles while the blood supply was maintained; mechanical stimulation was avoided. A portion of the muscle was frozen *in situ* by means of small brass clamps (freezing area approx. 6cm.^{2}) cooled in liquid N_2.

Determination of metabolites. Dihydroxyacetone phosphate and fructose 1,6-diphosphate were assayed spectrophotometrically by the method of Bücher & Hohorst (1963) and glyceraldehyde 3-phosphate was measured by the method of Racker (1957) with the following final concentrations: $Na_4P_2O_7$–HCl buffer, pH 8·8, 33mM; sodium arsenate, 3·3mM; NAD$^+$, 0·2mM; EDTA, 0·17mM; crystalline glyceraldehyde 3-phosphate dehydrogenase, 30μg./ml. Dihydroxyacetone phosphate and fructose 1,6-diphosphate could be measured in the same cuvette by adding in succession triose phosphate isomerase and aldolase (30μg./ml. of each).

When the concentration of the substance to be determined was less than 20μM, a fluorimeter was used in place of the spectrophotometer. In the fluorimetric assay it was essential to compare the fluorescence of the unknown with that of standard solutions at the same time. The instruments used were a Zeiss PMQII spectrophotometer and a Beckman ratio fluorimeter, fitted with Wratten filters no. 18A at the light-source and no. 2B and no. 44 before the photomultiplier. The concentrations of metabolites in tissues are expressed in terms of molarity on the assumption that 1g. of tissue equalled 1ml. The error in this assumption is unimportant in the present context.

Measurement of the equilibrium constants of triose phosphate isomerase and aldolase. The equilibrium constants of triose phosphate isomerase

$$K_{\text{TPI}} = [\text{DHAP}]/[\text{GAP}] \qquad (6)$$

and of aldolase

$$K_{\text{aldolase}} = [\text{DHAP}][\text{GAP}]/[\text{FDP}] \qquad (7)$$

at 38°, pH 7 and I 0·25 were measured in mixtures con-

taining various concentrations of substrates and crystalline rabbit muscle enzymes. When high enzyme concentrations were used, the suspensions of crystalline enzyme were centrifuged at 18000*g* and 4° for 40min. The supernatants were decanted and the precipitates dissolved in water to a convenient volume. The ionic strength of the incubation mixtures, including the $(NH_4)_2SO_4$ contained in the purified enzymes, was calculated from the formula $I = 0{\cdot}5\sum c_i z_i^2$ and it was adjusted to 0·25 with KCl. Sodium phosphate buffer, pH 7·0, was present at a final concentration of 10mM. $[H^+]$ was measured electrometrically at the end of the incubation. Equilibrium was approached from both directions. The mixture was incubated until the concentration ratio was constant, which was achieved in less than 10min. The reaction was stopped by addition of $HClO_4$ (final concn. 0·66M). After centrifugation, the pH of the deproteinized supernatants was taken to approximately 7 with 5M-KOH. The mixtures were kept on ice for 20–30min., after which dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and fructose 1,6-diphosphate were measured in the supernatants.

RESULTS

Equilibrium constants of the aldolase and triose phosphate isomerase reactions at 38° *and I* 0·25. The incubation mixtures contained 5 or 10μg. of rabbit muscle triose phosphate isomerase/ml., and 25 or 50μg. of rabbit muscle aldolase/ml. When fructose 1,6-diphosphate was the substrate, its initial concentration was 2·2–8mM; when dihydroxyacetone phosphate and glyceraldehyde 3-phosphate were substrates, their initial concentration was 0·38–1·3mM.

The equilibrium constants obtained (with S.E.M. from 30 observations) were:

$$K_{TPI} = [DHAP]/[GAP] = 22{\cdot}0 \pm 0{\cdot}246$$

$$K_{aldolase} = [DHAP][GAP]/[FDP] = 0{\cdot}99 \pm 0{\cdot}015 \times 10^{-4}\text{M}$$

These values were unaffected by pH variations between 6·35 and 7·12 or by the direction of approach to equilibrium. Addition of 5mM-Mg^{2+} had no effect on either constant, but with 50mM-Mg^{2+}, $K_{aldolase}$ decreased to $0{\cdot}56 \pm 0{\cdot}02 \times 10^{-4}$M (mean ± S.E.M. of four observations) (see Meyerhof, 1951; Holzer, 1956).

The equilibrium constants for the triose phosphate isomerase and aldolase reactions were also measured with enzyme and substrate concentrations similar to those in the tissues. For the triose phosphate isomerase reaction the equilibrium constant with 1μM rabbit muscle triose phosphate isomerase and glyceraldehyde 3-phosphate concentrations in the range 0·4–4μM was found to be identical with that estimated with low enzyme concentrations and large substrate concentrations, as described previously. For the aldolase reaction, the apparent equilibrium constant decreased with decrease of the substrate concentrations and increase of the enzyme concentration (Table 1), indicating binding of fructose 1,6-diphosphate by aldolase.

In experiments in which crystalline rabbit muscle aldolase and triose phosphate isomerase at

Table 1. *Effect of concentration of reactants on the [DHAP][GAP]/[FDP] ratio at equilibrium*

The incubation mixtures contained the substrate, 10mM-sodium phosphate buffer, pH 7, KCl to adjust *I* to 0·25, and aldolase at the concentrations indicated. Incubations were at 38°.

[Aldolase] (μM)	Incubation time (min.)	Final [DHAP] (μM)	Final [GAP] (μM)	Final [FDP] (μM)	$10^4 \times \frac{[DHAP][GAP]}{[FDP]}$ (M)
1	20	49·8	44·4	26·0	0·85
		30·2	27·5	8·9	0·93
		6·3	5·6	0·5	0·71
		3·3	2·7	0·2	0·45
1	25	54·0	45·6	24·6	1·00
		31·9	26·3	9·5	0·88
		6·2	5·3	0·6	0·55
		3·1	2·8	0·3	0·29
9	10	20·1	20·4	6·0	0·68
		8·8	10·1	1·7	0·52
		5·3	5·6	0·7	0·42
		2·9	2·8	0·2	0·41
9	20	19·4	19·1	5·8	0·64
		8·9	9·5	1·6	0·53
		5·3	5·2	0·6	0·45
		2·8	2·6	0·2	0·36

Table 2. *[DHAP]/[GAP] and [DHAP][GAP]/[FDP] ratios in the presence of rabbit muscle aldolase and triose phosphate isomerase*

The incubation mixtures contained aldolase, triose phosphate isomerase and fructose 1,6-diphosphate within the indicated concentration ranges and 10mM-sodium phosphate buffer, pH7·0. *I* was adjusted to 0·25 with KCl. Incubations were at 38° for 10–30min. The ratios in the last two columns are means ± S.E.M., with the number of observations in parentheses.

[Aldolase] (μM)	[TPI] (μM)	Initial [FDP] (μM)	Final [DHAP] (μM)	Final [GAP] (μM)	Final [FDP] (μM)	$\frac{[DHAP]}{[GAP]}$	$10^4 \times \frac{[DHAP][GAP]}{[FDP]}$ (M)
9	1	20–80	35–120	1·6–6·0	0·6–8·5	21·6 ± 0·64 (6)	0·85 ± 0·035 (6)
18	1	20–80	50–114	2·1–4·2	1·6–7·8	25·3 ± 1·73 (5)	0·66 ± 0·024 (5)
54	5	20	24–26	1·0–1·2	0·3–0·7	23·1 (2)	0·63 (2)

Table 3. *Dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and fructose 1,6-diphosphate concentrations in freeze-clamped liver and muscle*

The analyses were performed as described in the text. The values are means ± S.E.M.

Tissue	No. of observations	Metabolite content [DHAP] (μM)	[GAP] (μM)	[FDP] (μM)	$\frac{[DHAP]}{[GAP]}$	$10^4 \times \frac{[DHAP][GAP]}{[FDP]}$ (M)
Liver (normal fed rat)	8	27 ± 3	2·9 ± 0·2	11·1 ± 2·0	9·3	0·071
Liver (rat given iodoacetate)	2	290	19·1	278	15·2	0·2
Muscle (posterior femoral)	10	28 ± 41	2·3 ± 0·26	65 ± 9·0	12·2	0·01

concentrations approaching those in muscle were incubated together, the initial concentrations of fructose 1,6-diphosphate were chosen to lead to equilibrium concentrations of the triose phosphates approaching those of muscle. Table 2 shows that the [DHAP]/[GAP] ratio was very close to K_{TPI} in all cases. The [DHAP][GAP]/[FDP] ratio approached $K_{aldolase}$ only with the lowest concentrations of aldolase used and was significantly below $K_{aldolase}$ with the higher concentrations of the enzyme. As in the experiments with aldolase alone, this deviation indicates binding of FDP by aldolase.

Dihydroxyacetone phosphate glyceraldehyde 3-phosphate and fructose 1,6-diphosphate concentrations in rat liver and muscle tissue. The results of analyses of freeze-clamped tissue for the substrates of TPI and aldolase are given in Table 3. The substrate concentrations in liver and muscle from normal fed animals are low, and the concentration ratios differ significantly from the equilibrium values. Analyses of liver tissue from rats that were given iodoacetate (1m-mole/l. of body water) 15min. before being killed are also shown. As would be expected, inhibition of glyceraldehyde phosphate dehydrogenase results in considerable increases in the concentrations of all three substrates, and the concentration ratios for both reactions move towards the equilibrium values.

DISCUSSION

The value of 22·0 found for K_{TPI} at 35° and *I* 0·25 is in good agreement with the estimate of 24 at the same temperature and undefined ionic strength by Meyerhof & Junowicz-Kocholaty (1943) and the value of 22 at 25° calculated by Burton & Waley (1968) from initial-rate parameters. It agrees less well with the value of 28 at 38° and *I* 0·19 reported by Lowry & Passonneau (1964). For the aldolase reaction, the present estimate of $0{\cdot}99 \times 10^{-4}$M for the equilibrium constant at 38° and *I* 0·25 agrees well with previous estimates of $1{\cdot}19 \times 10^{-4}$M (Herbert, Gordon, Subramanyan & Green, 1940) and $0{\cdot}9 \times 10^{-4}$M (Lowry & Passonneau, 1964) at the same temperature.

Values of the [DHAP][GAP]/[FDP] ratios in rat liver and muscle (Table 3) are much lower than the equilibrium constant for the aldolase reaction, especially in the latter tissue. The [DHAP]/[GAP] ratio is also significantly lower than K_{TPI}. In view of the low concentrations of substrates present in the tissues, especially of glyceraldehyde 3-phosphate, a possible explanation of these apparent deviations from equilibrium is the assumption that significant proportions of the substrates exist in the enzyme-bound form. Approximate estimates of the concentrations of aldolase and triose phos-

Table 4. *Catalytic activity, turnover number and calculated concentrations of triose phosphate isomerase and aldolase in liver and muscle*

Enzyme activities are expressed as μmoles of substrate converted/min., at 25°, optimum pH and optimum substrate concentration. The turnover number is defined as μmoles of substrate converted/min./μmole of enzyme, under the same conditions. It was assumed that the turnover numbers of rat liver and rabbit muscle triose phosphate isomerases are the same, and that the turnover numbers of rat liver and rabbit liver aldolases are also the same. Where no reference is given the values were supplied by L. V. Eggleston (personal communication).

Tissue	Enzyme	Substrate	Enzyme activity (μmoles/min./g. wet wt. of tissue)	Turnover number (μmoles of substrate/min./ μmole of enzyme)	Calculated enzyme concentration (μM)	References
Rat liver	Triose phosphate isomerase	GAP	500		1	
Rat muscle			1830		4	Hohorst, Reims & Bartels (1962)
Rabbit muscle				500000		Burton & Waley (1966)
Rat liver	Aldolase	FDP	5·5		12	
Rabbit liver				460		Morse & Horecker (1968)
Rat muscle					45	Srere (1968)

phate isomerase in the tissues can be made from the maximum activities determined by assays and the turnover numbers of the enzymes. In the absence of turnover numbers for the enzymes of rat tissue, it is assumed that those of rabbit muscle triose phosphate isomerase and rabbit liver aldolase are applicable. The results of such calculations (Table 4) indicate that the enzyme concentrations are of the same order as the substrate concentrations. However, direct determination of the [DHAP]/[GAP] ratios with low substrate and high enzyme concentrations gave values close to K_{TPI}, showing that substrate binding by the enzymes is not responsible for the deviations from equilibrium found in the liver. The only other enzyme likely to bind glyceraldehyde 3-phosphate is glyceraldehyde phosphate dehydrogenase, but although this enzyme is present in higher concentrations in muscle (22 μM) than in liver (6 μM) the deviation from equilibrium in the triose phosphate isomerase system is, if anything, smaller in muscle than in liver. These considerations suggest that there is a true disequilibrium of the triose phosphate isomerase system in the liver of normal fed rats, and that the measured glyceraldehyde 3-phosphate content is a fair approximation of the amount of free glyceraldehyde 3-phosphate in both liver and muscle. This conclusion is borne out by the following calculations.

The net flux, v, through the triose phosphate isomerase reaction corresponding to the observed disequilibrium in liver tissue may be calculated from the steady-state rate equation for a reversible single-substrate reaction (Haldane, 1930):

$$v = \frac{V_f[GAP/K]^f_m - V_r[DHAP]/K^r_m}{1+[GAP]/K^f_m+[DHAP]/K^r_m} = \frac{\frac{V_f[GAP]}{K^f_m}\left(1-\frac{V_r K^f_m}{V_f K^r_m}\cdot\frac{[DHAP]}{[GAP]}\right)}{1+[GAP]/K^f_m+[DHAP]/K^r_m} \quad (8)$$

where V_f is the maximum rate of the reaction glyceraldehyde 3-phosphate → dihydroxyacetone phosphate and V_r that of the reverse reaction, and K^f_m and K^r_m are the Michaelis constants for glyceraldehyde 3-phosphate and dihydroxyacetone phosphate respectively.

The ratio $V_f K^r_m / V_r K^f_m$ is equal to the overall equilibrium constant for the reaction, K_{TPI} (Haldane, 1930). For the rabbit muscle enzyme, $K^f_m = 460\,\mu M$ and $K^r_m = 870\,\mu M$ (Burton & Waley, 1968). Similar values of K^f_m have been reported for the enzyme from several sources (Burton & Waley, 1968). For rat liver the second and third terms in the denominator of eqn. (8) may therefore be assumed to be negligible. Hence

$$v = \frac{V_f[GAP]}{K^f_m}\left(1-\frac{[DHAP]/[GAP]}{K_{TPI}}\right)$$

The second term in the brackets is the reciprocal of the 'steady state ratio', as defined by Waley (1964). From assays of triose phosphate isomerase in rat liver at 25° with saturating substrate concentration, $V_f = 500\,\mu$moles/min./g. (Table 4). With

$K_m^f = 460\,\mu M$, $K_{TPI} = 22{\cdot}0$, [GAP] $= 2{\cdot}9\,\mu M$ and [DHAP]/[GAP] $= 9{\cdot}3$ (Table 3), the calculated net flux is

$$v = \frac{500 \times 2{\cdot}9}{460}\left(1 - \frac{9{\cdot}3}{22{\cdot}0}\right)$$

$= 1{\cdot}8\,\mu$moles of dihydroxyacetone phosphate formed/min./g. of tissue.

The value of V_f was measured at 25°, and the rate at 38° may be about twice this value. However, triose phosphate isomerase is competitively inhibited by phosphate, with $K_i = 6$ mM (Burton & Waley, 1966), and since the P_i content of rat liver tissue is 6 mM (R. L. Veech, L. Raijman & H. A. Krebs, unpublished work), the rate will be halved by this inhibition. Thus the above flux may be taken as a reasonable approximation to the true value under physiological conditions.

The result of this calculation is in satisfactory agreement with the known rates of glycolysis (maximal rates 3·0 μmoles/min./g., H. F. Woods & H. A. Krebs, unpublished work) and of gluconeogenesis from lactate (maximal rates 2·3 μmoles/min./g.; Ross, Hems, Freedland & Krebs, 1967) in rat liver. The rate of flux through the triose phosphate isomerase system is at least as high as the rates of glycolysis and gluconeogenesis.

Entirely different types of experiments by Schambye, Wood & Popják (1954), Rose *et al.* (1962) and Hostetler, Williams, Shreeve & Landau (1969) also led to the conclusion that the triose phosphate isomerase reaction is not at equilibrium in the liver. These authors investigated the distribution of labelled carbon in glucose synthesized from glycerol, lactate and pyruvate and found that when glycerol is the precursor, more labelled carbon appeared in C-1, C-2 and C-3 of glucose than in C-4, C-5 and C-6. The reverse holds when lactate and pyruvate are the precursors. These findings can be satisfactorily explained by disequilibrium of the triose phosphate isomerase reaction and no alternative explanation is available.

In resting muscle, the steady-state ratio for the TPI reaction is closer to K_{TPI} in spite of the low concentration of glyceraldehyde 3-phosphate (Table 3); thus the flux must be very small.

The observed effects of substrate and enzyme concentrations on the apparent equilibrium constant of the aldolase reaction (Tables 1 and 2) indicate binding of fructose 1,6-diphosphate, but are not sufficient to explain the extent of deviation from equilibrium observed in the tissues, especially in muscle, where the steady-state ratio is smaller than $K_{aldolase}$ by a factor of 100. The disequilibrium may be due to inhibition by ATP, which is known to be a competitive inhibitor of muscle aldolase (Spolter, Adelman & Weinhouse, 1965) but not of liver aldolase.

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