EQUILIBRIA OF TWO-PARTNER REACTIONS OF ENERGY SUPPLYING METABOLISM IN MUSCLE

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Evidence has been given in previous communications (Hohorst et al. 1959, 1961) that it is possible to evaluate the steady state of some red/ox-reactions in the C-compartment of rat liver cells from the overall content (level) of metabolites in the tissue.

In this paper we present a further study on equilibria in living material with regard to some two-partner reactions of the Embden-Meyerhof-Krebs-pathway in striated muscle.

Methods and materials: The abdominal wall muscle of rats was freed from skin in a short ether anesthesia and cut along the median line. One half of the muscle layer was pressed between two aluminum blocks previously cooled with liquid air, separated from the body and immersed in liquid air. After pulverization of the tissue under liquid air, perchloric acid extracts were made and neutralized to pH 5-6 with KOH. In the extracts the following metabolites were estimated by means of enzymatic assays, which have been described elsewhere (Hohorst et al.1959, 1962): Glucose-6-phosphate (G_6P), fructose-6-phosphate (F_6P), glucose-1-phosphate (G_1P), dihydroxyacetonephosphate (DAP), glyceraldehyd-3-phosphate (GAP), 3-phosphoglycerate (3-PGA), 2-phosphoglycerate (2-PGA), phosphoenolpyruvate (PEP) and isocitrate. Citrate was estimated enzymatically with malate-dehydrogenase and purified citritase from Klebsiella pneumoniae,

which had been prepared according to a slight modification of the prescription given by Dagley and Dawes (1955). In the stimulation experiments the muscles were tetanized electrically in situ with immediately following fixation between cooled metal blocks. The activity of enzymes in the muscle was estimated in 0.1 M phosphate buffer extracts (pH 7.6) at 25°C according to Delbrück et al. 1959 and Pette 1961.

Results: In table 1 the activities of some glycolytic enzymes in muscle are shown:

Table 1

Enzyme	Activity (μMol/h/$g_{fr.w.}$)x10^{-3}
Phosphohexoseisomerase	13
Phosphoglucomutase	2.5 a)
Phosphotrioseisomerase	110
Phosphoglyceratemutase	21
Enolase	4.5

Level of glycolytic enzymes in abdominal wall muscle of the rat as tested in phosphate buffer extracts, pH 7.6, 25°C, under optimal conditions.

a) Considered as minimal value.

Comparing the values with the flow rate of glycolytic reactions in resting muscle, which was estimated as about 2 μMol/h/$g_{fr.w.}$ *), one may assume that none of these enzymes is rate limiting in vivo. Thus one may assume further that the corresponding reactions are close to the thermodynamic equilibrium. Since all equilibria considered here refer to two-partner reactions A $\rightleftharpoons$ B , their steady state in the tissue,

*) On the basis of 11 μatom/h/$g_{fr.w.}$ oxygen consumption (Zierler 1956) and assuming glucose solely to be oxidized.

which is given by the ratio $\frac{[B]}{[A]}$*) (i.e. the ratio of the true concentrations of reactants in the tissue**), therefore should be nearly equal to the corresponding equilibrium constants in each case.

The apparent equilibria $Q = \frac{\{B\}}{\{A\}}$ in the tissue were calculated from the levels of reactants as determined in resting muscle (table 2),

Table 2

Substance	Level	Substance	Level
G_6P	769 ± 100 (24)	3-PGA	37 ± 3 (11)
G_1P	47 ± 9 (5)	2-PGA	4.3 ± 1 (5)
F_6P	291 ± 51 (10)	PEP	13 ± 1 (11)
DAP	31 ± 3 (28)	citrate	147 ± 36 (8)
GAP	1.6 ± 1 (10)	isocitrate	12 ± 1 (4)

Levels of metabolites in resting abdominal wall muscle of the rat in mμMol/$g_{fr.w.}$ $\pm \frac{s}{\sqrt{n}}$. Number of assays in parentheses.

and compared with the corresponding equilibrium constants in table 3:

Table 3

A	Reaction	B	Q	$K'_{equil.}$
G_6P^{2-}	⟷	F_6P^{2-}	3.8×10^{-1}	4.3×10^{-1}
G_6P^{2-}	⟷	G_1P^{2-}	6.1×10^{-2}	5.5×10^{-2}
GAP^{2-}	⟷	DAP^{2-}	2.1×10^{1}	2.2×10^{1}
$3\text{-}PGA^{3-}$	⟷	$2\text{-}PGA^{3-}$	1.2×10^{-1}	1.7×10^{-1}
$2\text{-}PGA^{3-}$	⟷	$PEP+H_2O$	3.0	3.0
$citrate^{3-}$	⟷	$isocitrate^{3-}$	8.2×10^{-2}	8.0×10^{-2}

Apparent equilibria $Q = \frac{\{B\}}{\{A\}}$ in resting abdominal wall muscle as calculated from levels in table 2. Values for $K'_{equil.}$ were calculated from free energy data as compiled by Burton (1957) and refer to 25°C, pH 7, aqueous solution.

*) $[A]$: concentration of substance A in free solution
$\{A\}$: level (overall content) of substance A in the tissue (cf. Hohorst et al. 1959)

**) The term "concentration in the tissue" means the true concentration of a substance in its particular space of reaction in the tissue.

As shown the apparent equilibria Q agree fairly well in each case with the thermodynamic equilibria represented by $K'_{equil.}$. Even when the flow rate of glycolytic reactions was accelerated to about 5×10^3 $\mu Mol/h/g_{fr.w.}$ (i.e. approximately 1000 times the resting value) by electrical tetanisation of the muscle *in situ* only small deviations of the apparent equilibria were observed (table 4).

Table 4

	G_6P	F_6P	Q	
resting	.769	.291	3.8×10^{-1}	$K'_{equil.} = 4.3 \times 10^{-1}$
5 sec. tetanus	2.60	.775	3.0×10^{-1}	
10 sec. tetanus	4.49	1.27	2.8×10^{-1}	
	GAP	**DAP**	**Q**	
resting	1.6	31	2.1×10^{1}	$K'_{equil.} = 2.2 \times 10^{1}$
1 sec. tetanus	2.3	73	3.2×10^{1}	
.5 sec. tetanus +1 sec. relax.	4.6	228	5.0×10^{1}	
	3-PGA	**PEP**	**Q**	
resting	37	13	3.5×10^{-1}	$K'_{equil.} = 5.0 \times 10^{-1}$
1 sec. tetanus	63	19	3.0×10^{-1}	
.5 sec. tetanus +1 sec. relax.	127	25	2.1×10^{-1}	

Apparent equilibria Q in stimulated muscle. Levels in $m\mu Mol/g_{fr.w.}$, $K'_{equil.}$ from table 3. Muscles were tetanized *in situ* and fixed between cooled blocks as soon as possible or after an interval of ~1 sec.

As shown from table 4 the constancy of the Q-values is not a result of a constancy of the levels themselves. This and the fact that the Q-values are shifted against the direction of the flow of glycolytic reactions indicate that the apparent equilibria actually reflect the steady state of reactions in the tissue. Finally, these findings confirm the assumption made above that none of the enzymes of table 1 is rate limiting *in vivo*.

Thus the conclusions can be drawn:

1) The steady state equilibria of five two-partner reactions of the glycolytic chain and citric acid cycle are close to their thermodynamic equilibria both in resting and working muscle. This means that practically no energy is lost at these steps of intermediary metabolism.

2) The activity of the enzymes, catalyzing these reactions (as tested in vitro), is in the same order of magnitude as the enzymatic capacity in vivo.

3) Hence the finding that the apparent equilibria in the tissue are nearly equal to the corresponding equilibrium constants (table 3 and 4) means that, for each reaction considered here, the ratio of metabolite levels is practically equal to the ratio of concentrations in the tissue, i.e.

$$\frac{\{F_6P\}}{\{G_6P\}} \simeq \frac{[F_6P]}{[G_6P]}; \quad \frac{\{G_1P\}}{\{G_6P\}} \simeq \frac{[G_1P]}{[G_6P]}; \quad \frac{\{DAP\}}{\{GAP\}} \simeq \frac{[DAP]}{[GAP]} \quad \text{... etc.}$$

Thus further evidence is given that in certain cases steady state equilibria in tissue or cells can be evaluated from the overall content (level) of metabolites, despite the fact that the true concentrations of the equilibrium partners in the tissue cannot be determined by any known analytical method.

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