APPARENT UNBALANCE BETWEEN THE ACTIVITIES OF 6-PHOSPHOGLUCONATE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASES IN RAT LIVER

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SUMMARY. The ratio of activities of 6-phosphogluconate dehydrogenase/glucose-6-phosphate dehydrogenase measured in liver extracts of rats in lipogenic nutritional conditions is only 0.2, suggesting an apparent physiological unbalance between the two dehydrogenases of the hexosemonophosphate shunt. This potential unbalance is enhanced by the fact that TPNH is a more powerful competitive inhibitor of 6-phosphogluconate dehydrogenase than of glucose-6-phosphate dehydrogenase. Accordingly, a strong activation of 6-phosphogluconate dehydrogenase would be required for efficient functioning of this pathway, unless there is an alternative outlet for 6-phosphogluconate so far unrecognized in animal tissues.

Qualitative identification of enzymes of the hexosemonophosphate shunt known to be a major source of TPNH in animal tissues, has had little complement of quantitative studies on the relative capabilities of the enzymes assumed to be involved. Studying the adaptive changes in lipogenic conditions in rat liver of enzymes that can give TPNH, we have found that the ratio of activities of 6PGDH/G6PDH measured in extracts in standard optimal conditions is only 0.2. Moreover, if assayed at nearly physiological concentrations of substrates and TPN^+/TPNH ratios the above quotient can become as low as ca. 0.01. These observations do not support the widespread confidence in the hexosemonophosphate shunt in animal tissues as an unbranched pathway, in which the hydrolysis of the lactone makes physiologically irreversible the formation of 6PG.

Abbreviations: 6PGDH, 6-phosphogluconate dehydrogenase (EC 1.1.1.44); G6PDH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); CCE, citrate-cleavage enzyme (EC 4.1.3.8); ME, malic enzyme (EC 1.1.1.40); ICDH(TPN^+), isocitrate dehydrogenase TPN^+dependent (EC 1.1.1.42); GK, glucokinase (EC 2.7.1.2); HK, hexokinase (EC 2.7.1.1); GDH, glutamate dehydrogenase (EC 1.4.1.3); G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate.

Experimental. Male rats of about 150 g have been used under two different nutritional conditions: a) starvation, 2 days; b) lipogenic condition by feeding animals with a high glucose diet (1) for 3 days after 2 days of starvation. The livers were homogenized with 3 vols of 5 mM $KHPO_4$ - 1 mM dithioerythritol - 150 mM KCl - 5 mM $KHCO_3$ - 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$, adjusted to pH 7.0. After centrifugation at 30,000 g x 20 min at 4° the supernatant was used for assay of enzyme activities. The glutamate dehydrogenase remaining in the pellet was also estimated after extraction by resuspension in 10 times its volume of the above mixture, centrifugation at 1000 g x 5 min and incubation of the supernatant with 1% Triton X-100 for 5 min at room temperature.

Results and discussion. The activities in rat liver extracts of enzymes that can supply TPNH and certain enzymes otherwise related to lipogenesis in starved animals and in animals fed a high glucose diet are shown in Fig. 1. The activities of G6PDH, 6PGDH, and malic enzyme, as well as citrate cleavage enzyme and glucokinase, are markedly increased with the high glucose diet, confirming observations reported by Gibson (2) and Rudack et al. (3), while the TPN dependent isocitrate dehydrogenase decreases slightly, although significantly ($P<0.001$). Glutamate dehydrogenase activity is more than twice in the fasted that in the animals refed the high glucose diet, which may be in relation with the higher need for amino acid catabolism in the fasting animals and is consistent with the critical role of this enzyme in NH_4^+ metabolism.

Despite the adaptive increase of 6PGDH activity, the ratio 6PGDH/G6PDH goes down to 0.2 in the lipogenic conditions. When a series of metabolites and wide spectrum environmental factors were assayed with these two dehydrogenases, the only important physiological effector found was TPNH*. Surprisingly, it was found to be a stronger competitive inhibitor of 6PGDH than of G6PDH. As shown in Fig. 2, the Ki/Km ratios for reduced and oxidized TPN are 5 for G6PDH and 1.4 for 6PGDH. A similar Ki/Km value for purified 6PGDH has been independently obtained by Procsal and Holten (4). A lower ratio, 0.7,

* Other compounds tested with each of the dehydrogenases (in the presence of 0.1 M KCl, 50 µM TPN^+, and 50 µM 6PG or 100 µM G6P as appropriate) were: acetyl-CoA, palmityl-CoA, ATP, citrate, erythrose-4-P, fructosediphosphate, lauric, linoleic and linolenic acids, and dithioerythritol (1 mM); Mg^{2+} and Mn^{2+} (5 mM); EDTA (10 mM); Triton X-100 (0.2%); pH 6.5 to 7.5 with imidazole and triethanolamine buffers.

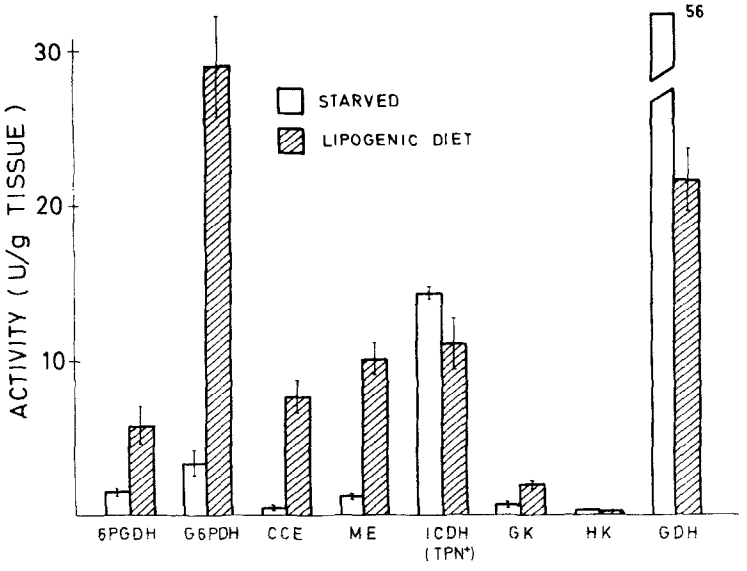

Fig. 1. Activities of enzymes in liver extracts from rats under starved and lipogenic conditions. The bars represent mean values ± S.D. for 8 rats. The increment in optical densities at 340 nm were measured in a Gilford 2400 spectrophotometer at room temperature. The reactions were started by addition of the respective substrates. 50 mM imidazole, pH 7.0, 5 mM $MgCl_2$ and about 600 µg of protein were used in all cases, except for GDH in which $MgCl_2$ was omitted. The following concentrations of the reaction components were used: 6PGDH: 0.5 mM 6PG, 0.25 mM TPN^+. G6PDH: 0.5 mM G6P, 0.25 mM TPN^+, and 0.3 U of 6PGDH. CCE: 10 mM citrate, 0.25 mM CoA, 5 mM ATP, 0.1 mM DPNH, 0.3 U malate dehydrogenase, 1 mM dithioerythritol. ME: 0.5 mM L-malate, 0.25 mM TPN^+, 1 mM dithioerythritol. ICDH(TPN^+): 0.5 mM DL-isocitrate, 0.25 mM TPN^+. GK: 100 mM glucose, 5 mM ATP, 0.25 mM TPN^+, 1 mM dithioerythritol and 0.3 U of G6PDH. HK: the same as for GK except that glucose was 0.5 mM. GDH: 10 mM α-ketoglutarate, 100 mM NH_4Cl, 0.1 mM DPNH, 1.5 mM ADP, and 5 mM EDTA.

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was obtained for this enzyme in extracts of starved rats (Fig. 3), possibly in relation with the occurrence of isoenzymes of 6PGDH in liver (5) whose proportion could change with the nutritional conditions.

If most of the 2.5 µmoles glucose/min/g tissue that can be phosphorylated in liver in lipogenic conditions (Fig. 1) were used for lipid synthesis, nearly 10 µmoles TPNH/min/g would be required. On the assumption that no less than 50% of the TPNH requirement has to

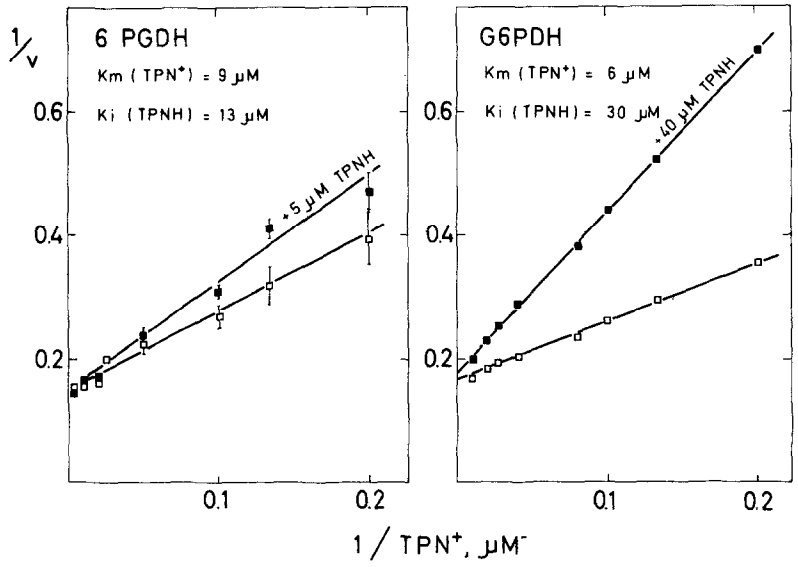

Fig. 2. Competitive inhibition by TPNH respect to TPN^+ of the activities of G6PDH and 6PGDH of rat liver in lipogenic conditions. Measurements were done in the same conditions as in Fig. 1 (using expanded recording scale: 0.1 O.D. full scale), except for the variable concentration of TPN^+, the presence of TPNH when indicated, and a lower concentration of protein: about 50 μg and 250 μg of protein for G6PDH and 6PGDH, respectively. The points whose standard deviation have been calculated are mean values obtained in 4-6 determinations. Those in which standard deviations have not been calculated are mean values of three determinations. The straight lines were obtained by regression analysis.

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be obtained from the hexosemonophosphate shunt, an activity in situ of up to 2.5 μmoles/min/g tissue for each of the two dehydrogenases would be needed. This value is well within the calculated possibilities of G6PDH, but too high by approximately one order of magnitude with respect to those calculated for 6PGDH at TPN^+/TPNH ratios and concentrations of substrates likely to prevail in vivo (Table I). Nevertheless, there is no accumulation of 6PG, although its level is significantly higher in lipogenic animals (Table II), presumably in relation with increased flux through this pathway. Accordingly, it seems that either liver 6PGDH can be markedly activated in situ by some as yet unrecognized factor(s) or there is an alternative pathway for 6PG in animal tissues.

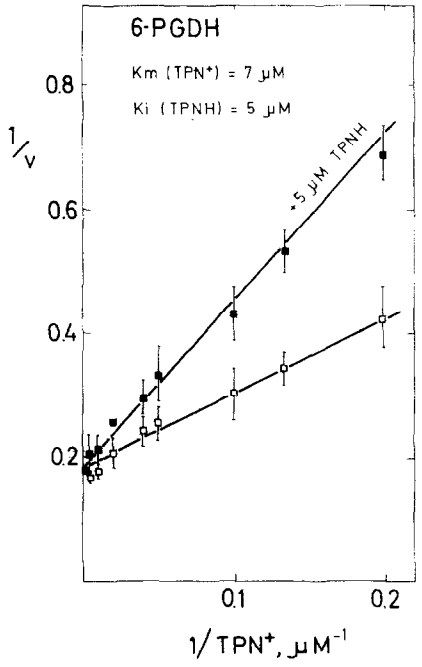

Fig. 3. Competitive inhibition by TPNH respect to TPN^+ of the activity of 6PGDH of liver extracts from starved rats. Conditions were as in Fig. 2. The straight lines were obtained by regression analysis.

The apparent physiological unbalance of about one order of magnitude between the two dehydrogenases of the hexosemonophosphate oxidative pathway in liver -and probably in certain other animal tissues*- throws in a note of caution on the widespread belief of an efficient working in sequence of these enzymes in a TPNH producing system, generally assumed to be controlled by the TPN^+/TPNH ratio (14). A purpose of this communication is to call attention to this problem, in the hope that observations from other laboratories could contribute to its solution.

* From values for the two dehydrogenases in other animal tissues recently reported it appears that the 6PGDH/G6PDH ratios may be as low as ca. 0.2 in guinea pig brain (9) and rat mammary gland (10), ca. 0.1 in human fibroblasts (11) and Schistosoma (12), and even ca. 0.01 in mouse unfertilized ovum (13).

Table I. Maximal rates and calculated activities of 6PGDH and G6PDH in extracts of liver from lipogenic and starved rats at physiological concentrations of substrates and coenzymes

	Ratio $TPN^+/TPNH$	Lipogenic 6PGDH units/g	Lipogenic G6PDH units/g	Lipogenic Ratio 6PGDH/G6PDH	Starved 6PGDH units/g	Starved G6PDH units/g	Starved Ratio 6PGDH/G6PDH
Maximal rates		5.8	29	0.2	1.5	3.4	0.44
Calculated activities	0.1	0.13	9.4	0.014	0.009	1.03*	0.009
	0.01	0.04	3.4	0.012	0.003	0.38*	0.008

Maximal rates were determined as indicated in Fig. 1. Calculation of the activities of 6PGDH and G6PDH at physiological concentrations of substrates and coenzymes were carried out with the equation derived by Wratten and Cleland (6) for enzymes with two substrates in the presence of a competitive inhibitor on the basis of the kinetic data and metabolite concentrations taken from Table II and Figs. 1, 2 and 3. Michaelis constants of 0.07 mM and 0.03 mM for 6PG and G6P, respectively, were used (4, 7). Ratios of $TPN^+/TPNH$ of 0.1 and 0.01, likely to cover the physiological range for free triphosphopyridine nucleotides in the cytoplasm, and a TPNH concentration of 0.25 mM (8), have been used for the calculations.

*Assuming Km and Ki values equal to those of the enzyme in lipogenic rats.

Table II. Concentrations of G6P and 6PG in liver of lipogenic and starved rats

	Lipogenic	Starved
nmoles G6P/g	187 ± 46	169 ± 61
nmoles 6PG/g	44 ± 15	31 ± 10

Results are mean values ± S.D. of 10 animals. Metabolites were estimated as Lagunas et al. (8) except that no anaesthesia was used.

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