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The content of pentose-cycle intermediates in liver in starved, fed *ad libitum* and meal-fed rats

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Liver content of pentose-cycle intermediates and the activity of the three major cytoplasmic NADPH-producing enzymes and pentose-cycle enzymes were measured in three dietary states: 48 h-starved rats, rats fed on a standard diet *ad libitum*, and rats meal-fed with a low-fat high-carbohydrate diet. Measured tissue contents of pentose-cycle intermediates in starved liver were: 6-phosphogluconate, 4.7 ± 0.5 nmol/g; ribulose 5-*P*, 3.7 ± 0.5 nmol/g; xylulose 5-*P*, 4.3 ± 0.4 nmol/g; sedoheptulose 7-*P*, 25.5 ± 1.3 nmol/g; and combined sedoheptulose 7-*P* and ribose 5-*P*, 30.6 ± 0.7 nmol/g. These values were in good agreement with values calculated from fructose 6-*P* and free glyceraldehyde 3-*P*, assuming the major transketolase, transaldolase, ribulose-5-*P* 3-epimerase and ribose-5-*P* isomerase reactions were all in near-equilibrium. Similar results were found in animals fed *ad libitum*. These relationships were not valid in animals fed on a low-fat high-carbohydrate diet, with tissue contents of metabolites in some cases being more than an order of magnitude higher than the calculated values. Measured tissue contents of pentose-cycle intermediates in these animals were: 6-phosphogluconate, 124.2 ± 13.9 nmol/g; ribulose 5-*P*, 44.8 ± 7.1 nmol/g; xylulose 5-*P*, 77.2 ± 9.4 nmol/g; sedoheptulose 7-*P*, 129.9 ± 10.1 nmol/g; and combined sedoheptulose 7-*P* and ribose 5-*P*, 157.0 ± 11.3 nmol/g. In all animals, regardless of dietary state, tissue content of erythrose 4-*P* was less than 2 nmol/ml. Liver activities of glucose-6-*P* dehydrogenase and 6-phosphogluconate dehydrogenase were increased from 3.5 ± 0.9 μmol/g and 7.3 ± 0.5 μmol/min per g in starved animals to 13.2 ± 1.1 and 10.5 ± 0.7 μmol/min per g in low-fat high-carbohydrate-fed animals. Despite these changes, the activities of transaldolase (3.4 ± 0.3 μmol/min per g), transketolase (7.8 ± 0.2 μmol/min per g) and ribulose-5-*P* 3-epimerase (7.5 ± 0.4 μmol/min per g) were not increased in meal-fed animals above those observed in starved animals (3.4 ± 0.2, 7.1 ± 0.3 and 8.6 ± 0.4 μmol/min per g respectively). The increase in the activity of oxidative pentose-cycle enzymes in the absence of any change in the non-oxidative pentose cycle appeared to contribute to the observed disequilibrium in the pentose cycle in animals meal fed on a low-fat high-carbohydrate diet.

INTRODUCTION

The work of Warburg & Christian (1931, 1932, 1936, 1937), Warburg *et al.* (1935), Lipmann (1936), and Dickens (1938*a*,*b*) in the middle 1930's led to the hypothesis that a second glucose-utilizing pathway existed. This pathway, the pentose cycle, was later described in the 1950's through the efforts of numerous laboratories (Horecker & Smyrniotis, 1951; Horecker *et al.*, 1951, 1954; Cori & Lipmann, 1952; Srere *et al.*, 1954; Gibbs & Horecker, 1954). The oxidative portion of the pentose cycle consists of reactions catalysed by glucose-6-*P* dehydrogenase (E.C. 1.1.1.49), 6-phosphoglucono-lactonase (EC 3.1.1.31) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) and supplies 2 molecules of NADPH for each molecule of glucose 6-*P* converted into ribulose-5-*P*. The non-oxidative portion of the pentose cycle consists of reaction catalysed by ribulose-5-*P* 3-epimerase (EC 5.1.3.1), ribose-5-*P* isomerase (EC 5.3.1.6), transketolase (EC 2.2.1.1) and transaldolase (EC 2.2.1.2) and catalyses the interconversion of fructose 6-*P* and glyceraldehyde 3-*P* with ribulose 5-*P*.

The pentose cycle is thought to play at least two important roles. Radioisotopic studies in liver (Wadke *et al.*, 1973; Lakshmanan & Veech, 1977), liver cells (Rognstad, 1980) and adipocytes (Flatt & Ball, 1964; Jungas, 1968; Rognstad & Katz, 1966) indicate that the pentose cycle supplies approximately half of the required cytoplasmic NADPH. The pentose cycle also supplies ribose 5-*P* for nucleotide synthesis (Dickens, 1938*b*; Marks & Feigelson, 1957). In addition, it has been proposed that the concentration of pentose-cycle intermediates affects both nucleotide synthesis (Pilz *et al.*, 1984; Boss, 1984) and glycolytic flux (Smith & Freedland, 1979; Sommercorn *et al.*, 1984).

Kauffman *et al.* (1969) showed that the brain contents of xylulose-5-*P* and ribulose-5-*P* were approximately the same as values calculated from the tissue contents of fructose-6-*P* and glyceraldehyde-3-*P*, assuming that the non-oxidative reactions of the pentose cycle were in near-equilibrium. Similarly it has recently been shown that, in rats fed on a standard diet *ad libitum*, the liver contents of xylulose 5-*P*, ribulose 5-*P*, sedoheptulose 7-*P*, ribose 5-*P* and presumably erythrose 4-*P* are described by near-equilibrium relationships dependent on the reactions of the non-oxidative pentose cycle and the liver content of fructose 6-*P* and free glyceraldehyde 3-*P*. The content of 6-phosphogluconate was related to the tissue content of ribulose 5-*P*, CO_2 and the free cytoplasmic $[NADP^+]/[NADPH]$ ratio (Casazza & Veech, 1986).

Despite the importance of this pathway *in vivo*, the effect of different dietary states on the enzymes and

substrates of this pathway are not well described. We have measured both pentose-cycle metabolites and pentose-cycle enzyme activities in starved, *ad lib.* fed and meal-fed animals in order to assess the validity of the equilibrium relationships defined in different dietary states.

MATERIALS AND METHODS

Enzymes

Transketolase (18 units/mg of protein), ribulose-5-*P* 3-epimerase (85 units/mg of protein) and ribose-5-*P* isomerase (765 units/mg of protein) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other enzymes were from Boehringer Mannheim (Indianapolis, IN, U.S.A.).

Reagents

Erythrose 4-*P*, xylulose 5-*P*, ribulose 5-*P*, ribose 5-*P*, sedoheptulose 7-*P*, NAD^+ and imidazole (grade III) were from Sigma. ATP, $NADP^+$ and NADH were from Boehringer Mannheim. Glutamate was from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Hydrazine hydrate was purchased from BDH Chemicals (Poole, Dorset, U.K.). All other chemicals were reagent-grade products.

Animals

Male Wistar rats (Charles River, Wilmington, MA, U.S.A.), weighing 200–225 g, were used. Three dietary states were studied: (1) animals fed on the standard N.I.H. rat ration *ad libitum* and then starved for 48 h; (2) animals fed on the standard N.I.H. rat ration *ad libitum* (5% fat, 23.5% crude protein and approx. 60% carbohydrate, largely as starch); and (3) animals starved for 48 h and then meal-fed with a high-carbohydrate low-fat diet (20.0% casein, 60.2% sucrose, 15% cellulose and 4.5% minerals and vitamins; Wooley & Sebrell, 1945). Animals were on a 12 h-light/12 h-dark cycle, with lights from 06:00 h to 18:00 h. Starved and *ad lib.*-fed animals were killed at 09:00 h and meal-fed animals at 12:00 h. Animals were killed by cervical dislocation, and livers were rapidly removed and frozen between two aluminium discs which had been cooled in liquid N_2 (Wollenberger *et al.*, 1960). $HClO_4$ extracts of frozen liver were prepared as previously described (Veech *et al.*, 1972), except that extracts were not treated with Florisil and were adjusted to pH 5.0–6.0 with KOH.

Enzyme assays

Liver homogenates were prepared as described by Veech *et al.* (1969) Glucose-6-*P* dehydrogenase and 6-phosphogluconate dehydrogenase (Rudack *et al.*, 1971) and malic enzyme (EC 1.1.1.40) activities (Hsu & Lardy, 1969) were assayed as described previously. Isocitrate dehydrogenase (EC 1.1.1.42) activity was measured in a reaction mixture containing 88 mM-imidazole, pH 8.0, 3.3 mM-$MgCl_2$ and 0.4 mM-$NADP^+$. Assays were initiated by addition of 0.5 mM-isocitrate. No significant reaction was observed before addition of isocitrate. Transketolase activity was measured in assays containing 100 mM-imidazole, pH 7.6, 2 mM-$MgCl_2$, 100 μM-thiamin pyrophosphate, 1 mM-$NADP^+$, 166 μM-xylulose 5-*P*, 3.5 units of glucose-6-*P* dehydrogenase and 7 units of phosphoglucoisomerase. Reactions were initiated by the addition of 0.2 mM-erythrose 4-*P*. No blank reaction was observed when either transketolase substrate, xylulose 5-*P* or erythrose 4-*P*, was omitted from the assay. Transaldolase was assayed by a modification of the method of Kauffman (1972). Assays contained 50 mM-imidazole, pH 7.6, 6 mM-$MgCl_2$, 5 mM-sodium arsenate, 1 mM-NAD^+, 20 mM-nicotinamide, 2 mM-sodium amytal, 200 μM-erythrose 4-*P* and 0.5 unit of glyceraldehyde-3-*P* dehydrogenase (EC 1.2.1.12)/ml. The blank reaction was observed and later subtracted from the observed rate. The reaction was initiated by the addition of 2 mM-fructose 6-*P*. No blank reaction was observed when fructose 6-*P* was the only substrate present. Ribulose-5-*P* 3-epimerase was assayed in incubations containing 100 mM-imidazole, pH 7.6, 0.5 mM-ribose 5-*P*, 0.5 mM-ribulose 5-*P*, 1 mM-$MgCl_2$, 0.2 mM-NADH, 0.1 mM-thiamin pyrophosphate, 20 mM-nicotinamide, 2 mM-sodium amytal, 1 mg of bovine serum albumin/ml, 0.6 unit of glycerol-phosphate dehydrogenase (EC 1.1.1.8)/ml, 90 units of triosephosphate isomerase (EC 5.3.1.1)/ml and 0.3 unit of transketolase/ml. Reactions were initiated by addition of liver extract. All enzyme assays were done at 38 °C. In all enzyme assays, substrate concentrations were such that enzyme activities were determined either at or near $V_{max.}$ conditions.

Metabolite assays

L-Lactate, malate, glucose, glucose 6-*P*, fructose-*P*, 6-phosphogluconate, isocitrate and ATP were measured as described by Lowry & Passonneau (1972). Dihydroxyacetone-*P* was measured as described by Bucher & Hohorst (1963). Xylulose 5-*P*, ribulose 5-*P* and combined sedoheptulose 7-*P* and ribose 5-*P* were assayed by a modification of the method of Kauffman *et al.* (1969), as described by Casazza & Veech (1986). Sedoheptulose 7-*P* was assayed as described by Racker (1974) with minor modification (Casaza & Veech, 1986). Erythrose 4-*P* was assayed as described previously (Casazza & Veech, 1986).

Metabolite recoveries were determined by adding a known amount of substrate to tissue before extraction with $HClO_4$. In each case the amount of substrate added was approximately equal to the tissue content of the substrate. Recoveries were calculated by taking the difference between substrate content/g of tissue in 'supplemented' tissue and that in 'non-supplemented' tissue and then dividing this by the amount of substrate added/g to the 'supplemented' tissue. This fraction was multiplied by 100 to give recoveries as percentages. Recoveries for xylulose 5-*P*, ribulose 5-*P* and combined sedoheptulose 7-*P* and ribose 5-*P* in starved tissue were 116, 96 and 116%, in *ad lib.*-fed animals 92, 107 and 97%, and in meal-fed animals 102, 102 and 96%. Recoveries for sedoheptulose 7-*P* in starved, *ad lib*-fed and meal-fed animals were 122, 112 and 99%. All other assays were done as described by Veech *et al.* (1972) and Veloso *et al.* (1972).

At low pH fructose-2,6-P_2 is rapidly converted into fructose 6-*P* and P_i (Van Schaftingen *et al.*, 1980). Subsequent reports of liver fructose-2,6-P_2 contents of 10–25 nmol/g in *ad lib.*-fed and re-fed animals (Neely *et al.*, 1981; Kuwajima & Uyeda, 1982; Van Schaftingen *et al.*, 1984) suggest that measurement of fructose 6-*P* in $HClO_4$ extracts may overestimate the liver content of fructose 6-*P*. Because of this, fructose 6-*P* was calculated from the tissue content of glucose 6-*P* and the phosphohexoisomerase equilibrium constant published by Lowry *et al.* (1964).

Free tissue glyceraldehyde 3-*P* was calculated from tissue dihydroxyacetone-*P* as described by Veech *et al.* (1979). The free cytoplasmic [NAD+]/[NADH] ratio was calculated as described by Williamson *et al.* (1967), by using an equilibrium constant of 1.11×10^{-11} for the lactate dehydrogenase reaction (Williamson *et al.* (1967). The free cytoplasmic [NADP+]/[NADPH] ratio was calculated as described by Veech *et al.* (1969), by using an equilibrium constant of 1.17 M for the isocitrate dehydrogenase reaction (Londesborough & Dalziel, 1968), 3.44×10^{-2} M for the malic enzyme reaction (Veech, 1968) and 1.72 M^{-1} for the 6-phosphogluconate dehydrogenase reaction (Villet & Dalziel, 1969).

All values are reported as means ± S.E.M. Statistical significance was determined by an unpaired Student's *t* test.

RESULTS AND DISCUSSION

Tissue contents of glycolytic and tricarboxylic-acid-cycle intermediates in starved, *ad lib.*-fed and meal-fed animals (Table 1) were similar to values previously reported (Lawson *et al.*, 1976; Veech & Guynn, 1974). With the exception of erythrose 4-*P*, significant changes were observed in almost all of the pentose-cycle intermediates measured in all three dietary states (Table 2). The increases in tissue contents of 6-phosphogluconate, ribulose 5-*P*, xylulose 5-*P*, sedoheptulose 7-*P* and combined sedoheptulose 7-*P* and ribose 5-*P* were particularly noteworthy. In these metabolites the changes observed between the *ad lib.*-fed and meal-fed states were significantly greater than the changes observed between the starved and *ad lib.*-fed states.

As previously reported (Veech *et al.*, 1969), the [NADP+]/[NADPH] ratio calculated from the isocitrate dehydrogenase redox couple was more reduced in starved animals than in *ad lib.*-fed and meal-fed animals (Table 3). The [NADP+]/[NADPH] ratio calculated from the malic enzyme redox couple also indicated that this ratio was more reduced in starved animals than in *ad lib.*-fed animals. Contrary to these results, the [NADP+]/[NADPH] ratio calculated from the 6-phosphogluconate dehydrogenase redox couple was more oxidized in starved animals than in meal-fed animals.

Concomitant with the increases in contents of liver pentose-cycle intermediates, glucose-6-*P* dehydrogenase was increased in meal-fed animals 3-fold above the value observed in starved animals (Table 4). The activity of 6-phosphogluconate dehydrogenase was also higher in meal-fed animals than in starved animals. Despite these changes, the observed transketolase and ribulose-5-*P* 3-epimerase activities were unchanged in starved, *ad lib.*-fed and meal-fed animals. Rather, transaldolase

Table 1. Measured liver metabolite contents in starved, *ad lib.*-fed and low-fat meal-fed animals

Metabolite content is reported as nmol/g wet wt. of tissue. Values are means ± S.E.M.; $n = 6$ in all cases. All other experimental details are described in the Materials and methods section.

Metabolite	Content (nmol/g)		
	Starved	*Ad lib.*-fed	Meal-fed
Glucose	5271 ± 199	7527 ± 179*	10213 ± 276‡§
P_i	4055 ± 256	3265 ± 205	3765 ± 138
2-Oxoglutarate	72 ± 9	243 ± 25*	279 ± 14‡
Isocitrate	19 ± 3	21 ± 1	23 ± 2
Malate	360 ± 32	261 ± 16†	640 ± 37‡§
Pyruvate	10 ± 1	112 ± 9*	243 ± 34‡§
Lactate	343 ± 34	701 ± 60*	1618 ± 5‡§
Acetoacetate	685 ± 35	161 ± 15*	23 ± 5‡§

* Significantly different from 'starved' value at $P < 0.01$.
† Significantly different from 'starved' value at $P < 0.05$.
‡ Significantly different from 'starved' value at $P < 0.01$.
§ Significantly different from '*ad lib.*' value at $P < 0.01$.

Table 2. Measured pentose-cycle intermediates in liver from starved, *ad lib.*-fed and meal-fed animals

Metabolite content is reported as nmol/g wet wt. of tissue. Values are means ± S.E.M.; $n = 6$ in all cases. All other experimental details are described in the Materials and methods section.

Metabolite	Content (nmol/g)		
	Starved	*Ad lib.*-fed	Meal-fed
Ribulose-5-*P*	3.7 ± 0.5	5.9 ± 0.3*	44.8 ± 7.1*†
Xylulose-5-*P*	4.3 ± 0.4	8.9 ± 0.4*	77.2 ± 9.8*†
Erythrose-4-*P*	< 2.0	< 2.0	< 2.0
Sedoheptulose-7-*P*	25.5 ± 1.3	34.0 ± 1.2*	129.7 ± 10.2*†
Sedoheptulose-7-*P* and ribose-5-*P*	30.6 ± 0.7	38.5 ± 1.6*	157.0 ± 11.3*†
6-Phosphogluconate	4.7 ± 0.7	9.0 ± 0.7*	124.2 ± 13.9*†
Glucose-6-*P*	62.2 ± 3.9	129.0 ± 9.3*	154.0 ± 9.9*†
Fructose-6-*P*	16.4 ± 1.0	41.3 ± 3.2*	55.7 ± 3.9*‡
Fructose-6-*P* (calc.)	18.6 ± 1.2	38.6 ± 2.2*	45.9 ± 3.0*
Glyceraldehyde-3-*P* (calc.)	0.67 ± 0.03	1.18 ± 0.06*	2.38 ± 0.11*†

* Significantly different from 'starved' value at $P < 0.01$.
† Significantly different from '*ad lib.*' value at $P < 0.01$.
‡ Significantly different from '*ad lib.*' value at $P < 0.05$.

Table 3. Redox states of NAD and NADP in liver from starved, *ad lib.*-fed and meal-fed rats

The metabolite values used in this table are taken from Tables 1 and 2. Values are means ± S.E.M.; $n = 6$ in all cases. All other experimental details are described in the Materials and methods section.

Redox couple	Starved	*Ad lib.*-fed	Meal-fed
$NADP^+/NADPH$			
Isocitrate dehydrogenase	0.0037 ± 0.0002	0.0112 ± 0.0002*	0.0122 ± 0.0008*
Malic enzyme	0.0101 ± 0.0012	0.0146 ± 0.0012‡	0.0126 ± 0.0011
6-Phosphogluconate dehydrogenase	0.0054 ± 0.0004	0.0043 ± 0.0003	0.0024 ± 0.0002*†
$NAD^+/NADH$ Lactate dehydrogenase	286.0 ± 31.2	1455 ± 62.2*	1345 ± 142

* Significantly different from 'starved' value at $P < 0.01$.
† Significantly different from 'starved' and '*ad lib.*' values at $P < 0.01$.
‡ Significantly different from 'starved' value at $P < 0.05$.

Table 4. Activities of liver enzymes bearing on the production of NADPH in starved, *ad lib.*-fed and low-fat meal-fed rats

Activites are reported as μmol of product produced/min per g wet wt. of liver. Assays were performed at 38 °C under the conditions described in the Materials and methods section. Values are means ± S.E.M.; $n = 6$ unless otherwise noted.

Enzyme	Activity (μmol/min per g)		
	Starved	*Ad lib.*-fed	Meal-fed
Glucose-6-*P* dehydrogenase	3.5 ± 0.9	3.4 ± 0.5	11.8 ± 0.9*‡
6-Phosphogluconate dehydrogenase	7.3 ± 0.5	8.6 ± 0.6	10.1 ± 0.4†
Isocitrate dehydrogenase	59.1 ± 1.5	55.8 ± 3.0	45.6 ± 1.8*
Malic enzyme	2.7 ± 0.1	3.5 ± 0.4	9.5 ± 0.7*‡
Transketolase	7.1 ± 0.3 ($n = 5$)	7.1 ± 0.2 ($n = 5$)	7.8 ± 0.2
Transaldolase	4.2 ± 0.2 ($n = 5$)	3.0 ± 0.3 ($n = 5$)*	3.4 ± 0.3†
Ribulose-5-*P* 3-epimerase	8.6 ± 0.4 ($n = 5$)	8.5 ± 0.3 ($n = 5$)	7.5 ± 0.4

* Significantly different from 'starved' value at $P < 0.01$.
† Significantly different from 'starved' value at $P < 0.05$.
‡ Significantly different from '*ad lib.*' value at $P < 0.01$.

Table 5. Equilibrium constants for the non-oxidative reactions of the pentose cycle under physiological conditions

All constants were determined at 38 °C, at pH 7.0, in the presence of 1 mM free $MgCl_2$, with the ionic strength of the solution adjusted to 0.25 M by the addition of KCl.

Enzyme system	Definition of constant	Value
Ribulose 5-*P* 3-epimerase (EC 5.1.3.1)	$K_1 = \frac{[\text{Xylulose 5-}P]}{[\text{ribulose 5-}P]}$	1.82
Ribose 5-*P* isomerase (EC 5.3.1.6)	$K_2 = \frac{[\text{Ribose 5-}P]}{[\text{ribulose 5-}P]}$	1.20
Transaldolase (EC 2.2.1.2)	$K_3 = \frac{[\text{Erythrose 4-}P][\text{fructose 6-}P]}{[\text{sedoheptulose 7-}P][\text{glyceraldehyde 3-}P]}$	0.37
Transketolase (EC 2.2.1.1)	$K_4 = \frac{[\text{Fructose 6-}P][\text{glyceraldehyde 3-}P]}{[\text{erythrose 4-}P][\text{xylulose 5-}P]}$	29.7
Transketolase (EC 2.2.1.1)	$K_5 = \frac{[\text{Ribose 5-}P][\text{xylulose 5-}P]}{[\text{sedoheptulose 7-}P][\text{glyceraldehyde 3-}P]}$	0.48

Table 6. Equilibrium expressions for pentose-cyclose intermediates in terms of [fructose 6-*P*] and [glyceraldehyde 3-*P*]

The constants are defined in Table 5.

$$[\text{Xylulose 5-}P] = [\text{glyceraldehyde 3-}P]^{\frac{1}{3}}[\text{fructose 6-}P]^{\frac{2}{3}}\,\frac{K_1^{\frac{1}{3}}K_5^{\frac{1}{3}}}{K_2^{\frac{1}{3}}K_3^{\frac{1}{3}}K_4^{\frac{1}{3}}}$$

$$[\text{Ribulose 5-}P] = [\text{glyceraldehyde 3-}P]^{\frac{1}{3}}[\text{fructose 6-}P]^{\frac{2}{3}}\,\frac{K_5^{\frac{1}{3}}}{K_1^{\frac{2}{3}}K_2^{\frac{1}{3}}K_3^{\frac{1}{3}}K_4^{\frac{1}{3}}}$$

$$[\text{Ribose 5-}P] = [\text{glyceraldehyde 3-}P]^{\frac{1}{3}}[\text{fructose 6-}P]^{\frac{2}{3}}\,\frac{K_2^{\frac{2}{3}}K_5^{\frac{1}{3}}}{K_1^{\frac{2}{3}}K_3^{\frac{1}{3}}K_4^{\frac{1}{3}}}$$

$$[\text{Sedoheptulose 7-}P] = \frac{[\text{fructose 6-}P]^{\frac{4}{3}}}{[\text{glyceraldehyde 3-}P]^{\frac{1}{3}}}\,\frac{K_2^{\frac{1}{3}}}{K_1^{\frac{1}{3}}K_3^{\frac{2}{3}}K_4^{\frac{2}{3}}K_5^{\frac{1}{3}}}$$

$$[\text{Erythrose 4-}P] = [\text{glyceraldehyde 3-}P]^{\frac{2}{3}}[\text{fructose 6-}P]^{\frac{1}{3}}\,\frac{K_2^{\frac{1}{3}}K_3^{\frac{1}{3}}}{K_1^{\frac{1}{3}}K_4^{\frac{2}{3}}K_5^{\frac{1}{3}}}$$

Table 7. Calculated values for pentose-cycle intermediates based on [fructose 6-*P*] and [glyceraldehyde 3-*P*] in starved, *ad lib.*-fed and meal-fed animals

Values are calculated by assuming near-equilibrium states for the reactions involved, as described in the Results and discussion section. Equilibrium constants are from Table 5. Liver contents of fructose 6-*P* and free glyceraldehyde 3-*P* are calculated from tissue contents of dihydroxyacetone-*P* and glucose 6-*P* as described in the Materials and methods section.

	Content (nmol/g)		
Metabolite	Starved	*Ad lib.*-fed	Meal-fed
Ribulose 5-*P*	1.4±0.1	2.7±0.2	3.8±0.1
Xylulose 5-*P*	2.5±0.1	4.9±0.3	6.9±0.2
Erythrose 4-*P*	0.17±0.01	0.31±0.02	0.53±0.01
Sedoheptulose 7-*P*	12.7±1.1	27.7±2.6	28.0±2.8
Sedoheptulose 7-*P* and ribose 5-*P*	14.4±1.8	30.9±2.8	32.5±2.9
Ribose 5-P	1.6±0.1	3.2±0.2	4.6±0.2

activity was slightly less in both *ad lib.*-fed and meal-fed animals than in starved animals.

The rate of liver fatty acid synthesis in animals meal fed on a low-fat high-carbohydrate diet is more than 60-fold higher than the rate observed in starved animals *in vivo* (Guynn *et al.*, 1972). These data suggest that the oxidative portion of the pentose cycle is subject to large changes in flux *in vivo*. This is further suggested by the increase in liver glucose-6-*P* dehydrogenase activity in animals meal-fed with a high-carbohydrate diet, reported by numerous groups (Fitch & Chiakoff, 1960; Veech *et al.*, 1969; Rudack *et al.*, 1971). The previously reported increases were in general greater than that reported here. Surprisingly, of the enzymes of the non-oxidative portion of the pentose cycle measured, no increase in activity was observed in meal-fed animals. These data suggest that in meal-fed animals the non-oxidative reactions of the pentose cycle may not be in near equilibrium with glycolytic intermediates, as previously shown in brain (Kauffman *et al.*, 1969) and in liver from animals fed on a standard diet *ad libitum* (Casazza & Veech, 1986).

When all of the non-oxidative reactions of the pentose cycle are in near equilibrium, the contents of pentose-cycle intermediates can be expressed as a function of the equilibrium constants of transaldolase, transketolase, ribulose-5-*P* 3-epimerase and ribose 5-*P* isomerase (Table 5) and tissue contents of fructose 6-*P* and free glyceraldehyde 3-*P* (Table 6). Therefore the agreement between measured values and values calculated from the tissue fructose 6-*P* and glyceraldehyde 3-*P* contents can be used as a criterion for near-equilibrium in the non-oxidative portion of the pentose cycle. In starved and *ad lib.*-fed animals the calculated values for xylulose 5-*P*, ribulose 5-*P* and sedoheptulose 7-*P* (Table 7) were 2–3-fold less than the measured values (Table 2). Calculated erythrose 4-*P* was well below the detection limits of the assay used for erythrose 4-*P*, 0.2 nmol/g, consistent with our inability to measure this metabolite. The agreement between calculated and measured values in both starved and *ad lib.*-fed animals, particularly considering the low contents of these metabolites and the possibility of significant substrate binding, suggests that in liver the content of pentose-cycle intermediates in both starved and *ad. lib*-fed animals is in near-equilibrium with liver fructose 6-*P* and free glyceraldehyde 3-*P*.

In meal-fed animals the calculated values for xylulose 5-*P*, 6.9±0.2 nmol/g, and ribulose 5-*P*, 3.9±0.1 nmol/g (Table 7), were more than an order of magnitude less than the measured values for these metabolites, 77.2±9.8 and 44.8±7.1 nmol/g (Table 2). Calculated sedoheptulose 7-*P* was more than 4-fold less than the measured value. These data suggest at least one of the non-oxidative

reactions of the pentose cycle is significantly displaced from equilibrium. The agreement between the [xylulose 5-*P*]/[ribulose 5-*P*] ratio in starved (1.21 ± 0.08), *ad lib.*-fed (1.50 ± 0.03) and meal-fed animals (1.77 ± 0.07) and the equilibrium constant for the ribulose-5-*P* 3-epimerase reaction, 1.82 (Table 5), indicates that this reaction is in near-equilibrium in all three dietary states. Similarly the ribose 5-*P* values, obtained by subtracting sedoheptulose 7-*P* values from combined ribose 5-*P* and sedoheptulose 7-*P*, in starved animals (5.1 ± 1.0 nmol/g), in *ad lib.*-fed animals (4.5 ± 1.7 nmol/g) and in meal-fed animals (27.1 ± 3.8 nmol/g), although less accurate than direct measurement, gave a range of values for the [ribose 5-*P*]/[ribulose 5-*P*] ratio of between 1.6 ± 0.4 in starved animals and 0.64 ± 0.09 in meal-fed animals, suggesting that the ribose-5-*P* isomerase reaction was also in equilibrium in all three dietary states. Because of our inability to measure erythrose 4-*P* in liver extracts, of the transketolase and transaldolase reactions, only the [xylulose 5-*P*][ribose 5-*P*]/[sedoheptulose 7-*P*][glyceraldehyde 3-*P*] ratio could be calculated. The ratios obtained, 1.4 ± 0.4 in starved and 1.0 ± 0.4 in *ad lib.*-fed animals, were only 2- and 3-fold higher than the equilibrium constant, 0.48. In meal-fed animals the calculated value was 7.0 ± 1.3, 15-fold greater than the equilibrium constant, indicating a significant disequilibrium in at least one of the transketolase reactions thought to play an important role in the pentose cycle.

Although the [$NADP^+$]/[NADPH] ratios as calculated from the isocitrate dehydrogenase, malic enzyme and 6-phosphogluconate dehydrogenase redox couples were in reasonable agreement in starved and *ad lib.*-fed animals, contrary to the other redox couples, the [$NADP^+$]/[NADPH] ratio as calculated from the 6-phosphogluconate dehydrogenase redox couple decreased in meal-fed animals. Whether these data indicate a true change in the cytoplasmic redox state is not clear. These data could also reflect a slight disequilibrium in the 6-phosphogluconate dehydrogenase reaction, a significant disequilibrium in the 6-phosphogluconolactonase reaction such that measured 6-phosphogluconate represented 6-phosphogluconate and a significant amount of the 6-phosphogluconolactone or a significant change in the ratio of bound/free 6-phosphogluconate or ribulose 5-*P* at different concentrations of these substrate. At present it is not possible to differentiate between these possibilities.

The control of the rate of flux through the pentose cycle in liver is not well understood, but the data presented here indicate that the activity of the enzymes of the non-oxidative portion of the pentose cycle are sufficient to maintain near-equilibrium conditions in starved and *ad lib.*-fed animals. As a result, the contents of pentose-cycle intermediates and fructose 6-*P* and glyceraldehyde 3-*P* are interdependent in these animals. This is not the case in animals meal-fed with a low-fat high-carbohydrate diet. Under these circumstances the near-equilibrium relationships previously defined in liver (Casazza & Veech, 1986) are not valid, and pentose-cycle intermediates are increased as much as 25-fold above the values observed in starved animals. The effect of this change on the overall metabolism of the liver is yet to be described.

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