Hepatic phosphoribosyl pyrophosphate concentration

Regulation by the oxidative pentose phosphate pathway and cellular energy status

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Measurements have been made of the tissue content of phosphoribosyl pyrophosphate (*PPRibP*) and of a range of metabolic intermediates involved in the energy charge of the cell, the glycolytic and pentose phosphate pathways, and of the activity of the enzymes of the pentose phosphate pathway and of *PPRibP* synthetase (EC 2.7.6.1) in the livers of normal, diabetic, insulin-treated diabetic and starved rats and in livers of rats previously starved and then re-fed with high-fat or high-carbohydrate diets. Diabetes, starvation and high-fat diet all caused a fall in the hepatic *PPRibP* content, whereas insulin treatment and high-carbohydrate diet raised the tissue content. A positive correlation was shown between the *PPRibP* content and ATP, energy charge and the cytosolic $[NAD^+]/[NADH]$ quotient. A positive association between the *PPRibP* content and the flux of glucose through the pentose phosphate pathway and the synthesis of ribose 5-phosphate via the oxidative enzymes of that pathway, including ribose-5-phosphate isomerase (EC 5.3.1.6), was also observed. A negative correlation was found between the ADP, AMP and P_i contents, and no correlation existed between *PPRibP* content and the enzymes of the non-oxidative branch of the pentose phosphate pathway. There was no correlation between hepatic *PPRibP* content and the activity of *PPRibP* synthetase measured *in vitro*. These results are considered in relation to the control of *PPRibP* synthetase in the liver *in vivo*.

INTRODUCTION

Phosphoribosyl pyrophosphate (*PPRibP*) is a primary substrate in nucleotide synthesis by both the 'de novo' and the salvage pathways, and is itself an activator of amidophosphoribosyltransferase (EC 2.4.2.14), the first enzyme of the 'de novo' pathway of synthesis (Becker *et al.*, 1979; Pilz *et al.*, 1984), and of carbamoyl-phosphate synthetase (glutamine) (EC 6.3.5.5), the isoenzyme involved in pyrimidine synthesis (Tatibana *et al.*, 1982).

PPRibP is made solely by *PPRibP* synthetase (EC 2.7.6.1), an enzyme which is subject to a complex system of control. It is dependent on ribose 5-phosphate availability, cell energy levels and end-product inhibition, and both P_i and bivalent cations are absolute requirements for activity, P_i having a stimulatory effect over a broad range of concentrations (Fox & Kelley, 1971; Becker *et al.*, 1979; Raivio *et al.*, 1981). Of the substrates for *PPRibP* synthetase, Atkinson & Fall (1967) have drawn attention to the role of the 'energy charge' in the regulation of the enzyme. There is also evidence, albeit controversial, that the availability of ribose 5-phosphate is also a crucial factor in regulating the tissue *PPRibP* content. In fibroblasts (Raivio *et al.*, 1981), cultured liver cells (Bashkin & Sperling, 1978) and liver slices (Boer *et al.*, 1976), ribose 5-phosphate does not appear to be limiting for *PPRibP* formation, except when *PPRibP* synthetase is activated by high concentrations of P_i , this conclusion being largely based on the failure of Methylene Blue to allow increased *PPRibP* synthesis despite measured increases in the flux through the

oxidative pentose phosphate pathway. On the other hand, Buchanan *et al.* (1982) have shown that when the pentose phosphate pathway is accelerated by the inclusion in the incubation media of hydrogen acceptors which do not, simultaneously, compromise the production of ATP, then the rate of formation of ribose 5-phosphate is limiting for the production of *PPRibP* in 3T6 fibroblasts. Further, Pilz *et al.* (1984) have shown that there is a parallel increase in *PPRibP* and cellular ribose 5-phosphate concentrations when cell cultures are exposed to increasing glucose concentrations. In addition, a significant correlation has been shown to exist between the enzymes of the pentose phosphate pathway (Steer *et al.*, 1985), the flux of glucose through the oxidative pentose phosphate pathway (Steer *et al.*, 1982, 1985) and accelerated growth in the early renal hypertrophy of diabetes. Thus, although considerable evidence exists, largely based on experiments involving Methylene Blue, which suggests that the oxidative branch of the pentose phosphate pathway is only minimally involved in the regulation of *PPRibP* availability, other evidence, based on less controversial hydrogen acceptors [amino acid synthesis, ascorbic acid synthesis (Buchanan *et al.*, 1982) or phenazine methosulphate (Steer *et al.*, 1982)] suggests that the supply of ribose 5-phosphate via the two dehydrogenases of the pentose phosphate pathway is crucial.

Two previous studies concerned with the regulation of *PPRibP* concentration in tissues from normal and diabetic rats have emphasized, again, the difficulties of relating the tissue concentration of *PPRibP* to rates of

Abbreviation used: *PPRibP*, phosphoribosyl pyrophosphate.

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growth (Kunjara *et al.*, 1986a,b,c). Thus diabetes produces opposite effects in mammary gland and kidney. In the mammary gland there is a diminution of the rates of milk production and protein synthesis, and of RNA content, and the *PPRibP* concentration falls (Kunjara *et al.*, 1986b). In the kidney, on the other hand, diabetes results in an initial hypertrophy, with an increased rate of protein and nucleic acid accretion, but, despite this contrast, the *PPRibP* concentration behaves in a similar fashion in the two tissues in that there is a fall in both cases (Kunjara *et al.*, 1986a,b). This apparent paradox of wasting in the mammary gland on the one hand, and accelerated growth processes in the kidney on the other, both being associated with a fall in tissue concentration of *PPRibP* in diabetes led to the present investigation of the hepatic response to diabetes.

The effects of diabetes, insulin, high- and low-carbohydrate diets and starvation on the hepatic concentration of *PPRibP* and on *PPRibP* synthetase have been measured. There is a substantial background of knowledge of the changes occurring in the alternative pathways of glucose metabolism, the redox state of the cell compartments, the energy charge of the cell and the metabolite profile under these conditions (Krebs & Veech, 1969; Greenbaum *et al.*, 1971; Casazza & Veech, 1986a), against which to examine the alterations in *PPRibP* concentration in order to gain further insight into factors, in particular the role of insulin, in the regulation of this key precursor of nucleotide synthesis.

METHODS

Animals

The rats used were of the Wistar strain weighing, at the outset of the experiment, 200–220 g.

Experimental treatments

Induction of diabetes. Experimental diabetes was induced by the intravenous injection of streptozotocin (60 mg/kg body wt.). Insulin, when appropriate, was given subcutaneously as either 2 or 5 i.u. (Monotard; Novo) daily for 3 days.

Dietary regimes. The composition of the diets given and the mode of administration of the diets were as described by Greenbaum *et al.* (1971). Starved rats were allowed free access to water.

The animals were killed by cervical dislocation at the times stated in the Tables.

Analytical procedures

Measurement of the tissue content of metabolic intermediates. Except for *PPRibP*, these measurements were made on HClO_4 extracts of freeze-clamped livers by standard procedures as described in Bergmeyer (1974) and in Greenbaum *et al.* (1971). The extraction and measurement of *PPRibP* were as previously described (Kunjara *et al.*, 1986a). Xylulose 5-phosphate was measured by the method of Racker, as described in Bergmeyer (1974).

Measurement of the activity of the enzymes of the pentose phosphate pathway. The activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), ribose-5-phosphate isomerase (EC 5.3.1.6), ribulose-

phosphate 3-epimerase (EC 5.1.3.1), transketolase (EC 2.2.1.1) and transaldolase (EC 2.2.1.2) were measured in the high-speed supernatant of a 1:10 (w/v) liver suspension in 0.25 M-sucrose medium buffered with 0.02 M-triethanolamine, pH 7.4, and containing 0.12 mM-dithiothreitol, by the methods described by Novello & McLean (1968).

RESULTS

Effect of diabetes, insulin and diet on hepatic *PPRibP* concentration and *PPRibP* synthetase activity

The hepatic concentration of *PPRibP* was significantly decreased in diabetes and in states with a depressed intake of carbohydrate, i.e. starvation for 3 days or starvation followed by re-feeding with a high-fat diet. In diabetes, the fall in *PPRibP* concentration was most marked in the 20-day-diabetic group (–31%); there was evidence that this effect was progressive with duration of diabetes, as shown by the smaller (–17%) decrease in *PPRibP* concentration seen 5 days after treatment with streptozotocin (Table 1). The activity of *PPRibP* synthetase showed only minor changes, and in the 20-day-diabetic rat was only 9% less than the control value (Table 1). After starvation for 3 days, the declines in *PPRibP* and *PPRibP* synthetase were approximately parallel; however the administration of a high-fat diet to starved rats produced the most pronounced fall in *PPRibP* concentration (–38%) seen in these present experiments. There was no parallel fall in *PPRibP* synthetase (Table 1).

The effect of starvation followed by re-feeding with a high-carbohydrate diet was to cause a massive overshoot in the hepatic concentration of *PPRibP* (+60%). This overshoot in *PPRibP* concentration was not accompanied by any increase in *PPRibP* synthetase activity; indeed, there was a small, but significant (–14%), decrease in activity per g of liver. Diabetic rats treated for 3 days with a low dose of insulin (2 i.u./day) showed little restoration of *PPRibP* or *PPRibP* synthetase activity (Table 1). As shown in Table 1, this dose of insulin was insufficient to restore normoglycaemia over a 24 h period. A higher dose of insulin (5 i.u./day) restored normoglycaemia and the *PPRibP* content of the liver. There was no correlation between the activity of *PPRibP* synthetase activity per g of liver and the *PPRibP* concentration.

Table 1 also shows the changes in total liver weight and total hepatic RNA. There is a severe loss of RNA from liver in starved rats and in the starved group re-fed with the high-fat diet; there is a substantial restoration of RNA synthesis in the group re-fed with the high-carbohydrate diet. Treatment of diabetic rats with high doses of insulin also led to a significant elevation of the hepatic RNA content (Table 1).

Correlation between hepatic *PPRibP* and energy status of the cell

The results shown in Fig. 1 illustrate that a positive correlation exists between the hepatic concentration of ATP, the energy charge of the cell and the *PPRibP* concentration ($r = 0.95$ and 0.94 respectively). These results are in accord with those of Atkinson & Fall (1967) on the properties of *PPRibP* synthetase isolated from *Escherichia coli*.

Table 1. Effects of diabetes, starvation and high-carbohydrate and high-fat diets on *PPRibP* concentration and *PPRibP* synthetase activity in rat liver

Results are given as means $\pm$ s.e.m. The initial weight of the animals in each group was 200–225 g in Group A and 190–200 g in Group B. Fisher's *P* values are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

	No. of animals	Final body wt. (g)	Liver wt. (g)	<i>PPRibP</i> content (nmol/g)	<i>PPRibP</i> synthetase activity (μ mol/h per g of tissue at 37 °C)	Total liver RNA (mg/liver)	Blood glucose (mM)
(A) Effect of diabetes and insulin treatment							
Diabetes 5 days							
Control (C)	6	231 $\pm$ 4	10.7 $\pm$ 0.33	10.6 $\pm$ 0.82	22.7 $\pm$ 1.3	110 $\pm$ 2.5	7.4 $\pm$ 0.8
5-day diabetic (D)	6	205 $\pm$ 4	9.49 $\pm$ 0.29	8.81 $\pm$ 0.58	18.8 $\pm$ 1.3	99 $\pm$ 2.6	28.0 $\pm$ 2.1
D/C (%)		89***	89***	83	82	90*	378***
Diabetes 20 days $\pm$ insulin							
Control (C)	11	266 $\pm$ 3	12.3 $\pm$ 0.57	10.5 $\pm$ 0.64	21.5 $\pm$ 0.7	139 $\pm$ 1.6	7.6 $\pm$ 0.4
20-day diabetic (D)	16	207 $\pm$ 8	11.5 $\pm$ 0.65	7.20 $\pm$ 0.32	19.1 $\pm$ 0.6	129 $\pm$ 2.5	27.6 $\pm$ 1.2
D/C (%)		78***	93	69***	91*	93*	363***
20-day diabetic with 3 days insulin (2 i.u./day) (D+I)							
D+I/D (%)	7	207 $\pm$ 7	13.2 $\pm$ 0.49	8.03 $\pm$ 0.84	20.7 $\pm$ 0.6	137 $\pm$ 4	22.2 $\pm$ 2.2
D+I/C (%)		100	115**	112	108	106	80*
		78***	107	77*	96	98	292***
20-day diabetic with 3 days insulin (5 i.u./day) (D+I)							
D+I/D (%)	6	215 $\pm$ 8	15.2 $\pm$ 1.1	12.1 $\pm$ 1.36	18.8 $\pm$ 0.8	160 $\pm$ 4.1	6.2 $\pm$ 1.3
D+I/C (%)		104	133**	168**	98	124***	22***
		81***	123*	115	87*	115***	82
(B) Effect of 3 days starvation followed by re-feeding with high-carbohydrate or high-fat diets							
Control (C)							
	18	214 $\pm$ 1	11.4 $\pm$ 0.24	12.4 $\pm$ 0.37	20.7 $\pm$ 0.04	136 $\pm$ 7	—
3-day starvation (S)							
S/C (%)	6	146 $\pm$ 3	5.0 $\pm$ 0.20	10.0 $\pm$ 0.61	14.6 $\pm$ 0.81	64.3 $\pm$ 3.4	—
		68***	44***	81**	71***	47***	—
3-day starvation + 3-day high-carbohydrate diet (S+CHO)							
S+CHO/S (%)	6	153 $\pm$ 3	10.8 $\pm$ 0.48	19.8 $\pm$ 2.2	17.1 $\pm$ 0.65	104 $\pm$ 5.6	—
S+CHO/C (%)		105	216***	198***	117*	162***	—
		71***	95	160***	83***	76**	—
3-day starvation + 3-day high-fat diet (S+F)							
S+F/S (%)	6	163 $\pm$ 3	8.47 $\pm$ 0.39	7.7 $\pm$ 0.83	20.7 $\pm$ 0.27	83 $\pm$ 4.1	—
S+F/C (%)		112*	169***	77*	142***	130**	—
		76***	74***	62***	100	61***	—

Fig. 1. Correlation between the hepatic *PPRibP* concentration and ATP (●) and energy charge (EC; △), free cytosolic $[NAD^+]/[NADH]$ (●) and $[NADP^+]/[NADPH]$ (○) in different dietary and hormonal conditions

The values given are the means (of not less than six values); the vertical lines represent twice the S.E.M. Where no lines are given, the S.E.M. is too small to show. The free cytosolic $[NAD^+]/[NADH]$ values were calculated from the pyruvate and lactate, and the free cytosolic $[NADP^+]/[NADPH]$ from the pentose phosphate and 6-phosphogluconate, contents of the liver (Krebs & Veech, 1969; Casazza & Veech, 1986b).

Also shown in Fig. 1 is the highly significant positive correlation ($r = 0.98$) between the hepatic *PPRibP* concentration and the free cytosolic $[NAD^+]/[NADH]$ quotient calculated from the pyruvate/lactate concentration ratios found in the liver in these experimental conditions.

Conversely, the data in Fig. 2 illustrates the negative correlation found between the hepatic *PPRibP* concentration and the ADP and AMP concentrations ($r = -0.97$ and -0.96 respectively), again in accord with Atkinson & Fall (1967). It is noteworthy that there is also a strong negative correlation between hepatic *PPRibP* and P_i concentrations in the hormonal and dietary conditions studied here ($r = -0.93$).

Correlation between the pentose phosphate pathway and the hepatic concentration of *PPRibP*

Three parameters linked to the functional activity of the pentose phosphate pathway were examined in relation to their possible regulatory role in *PPRibP* formation. These are: (i) the flux of glucose through the

pentose phosphate pathway as measured by the $^{14}CO_2$ yields from C-1 and C-6 of glucose (C_1-C_6); (ii) the concentration of the intermediates of the pathway, 6-phosphogluconate, pentose phosphates and the free cytosolic $[NADP^+]/[NADPH]$ ratio; and (iii) the activities of the enzymes of the oxidative and non-oxidative routes leading to pentose phosphate and ribose 5-phosphate formation. These results are given in Figs. 1 and 3 and in Table 2.

The free cytosolic $[NADP^+]/[NADPH]$ ratio, calculated from the xylulose 5-phosphate and 6-phosphogluconate concentrations by using the equilibrium constants determined by Casazza & Veech (1986b), was linearly correlated with the hepatic *PPRibP* concentration ($r = 0.98$) (Fig. 1). A positive correlation was also found between the hepatic concentration of *PPRibP* and the flux of glucose through the pentose phosphate pathway, the 6-phosphogluconate and xylulose 5-phosphate concentrations. However, the relationship between *PPRibP* concentration and the above parameters was logarithmic rather than linear (see Fig. 3b).

Fig. 2. Correlation between the hepatic *PPRibP* concentration and ADP, AMP and P_i in different dietary and hormonal conditions

The values given are the means (of not less than six values); the vertical lines represent twice the S.E.M.

As for the enzymes involved in the oxidative route to ribose 5-phosphate formation, there was evidence for a positive association between the activity of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and ribose-5-phosphate isomerase with the hepatic concentration of *PPRibP* (Table 2). On the other hand, no association was found between the activities of the non-oxidative route to pentose phosphate formation, namely transketolase, transaldolase and pentose phosphate epimerase, and *PPRibP* concentration in the livers of these groups of rats (Table 2).

DISCUSSION

The present experiments do not permit a direct evaluation of the contribution of the relative rates of *PPRibP* synthesis and degradation to the steady-state concentration. However, an increased biosynthesis and bioavailability, and more rapid utilization, of *PPRibP* for nucleotide synthesis is indicated by the increases, greater than in the corresponding control rats, in the total hepatic RNA over the experimental period in the high-insulin and high-carbohydrate states. In each of these groups, the direction of change of the steady-state *PPRibP* concentration paralleled the change in requirement for nucleotides for RNA synthesis. Thus in the following discussion the assumption is made that, in the present conditions, in the liver a rise in the measured

concentration of *PPRibP* equates with an increase in the synthesis of this compound.

In the present experiments there is evidence for an increase in the supply of the required substrates, ribose 5-phosphate via the pentose phosphate pathway and ATP via glycolytic flux, both of which appear to play a regulatory role in the control of *PPRibP* synthesis; the apparently lesser role played by P_i is also considered.

Energy charge and P_i

The high positive correlation between the *PPRibP* concentration and ATP and the $[NAD^+]/[NADH]$ quotient [a parameter reflecting the rate of glycolysis and ATP production (Krebs & Veech, 1969; Veech *et al.*, 1970; Greenbaum *et al.*, 1971)] (Fig. 1) and the negative correlation with respect to ADP and AMP (Fig. 2) implies a significant regulatory role for the energy charge on *PPRibP* synthesis and is consistent with the known inhibitory action of ADP on *PPRibP* synthetase (Atkinson & Fall, 1967; Becker *et al.*, 1979).

An unexpected aspect of the present work, in view of the many observations stressing the importance of P_i as an activator of *PPRibP* synthetase (see Hershko *et al.*, 1969; Fox & Kelley, 1971; Planet & Fox, 1976; Bashkin & Sperling, 1978; Becker *et al.*, 1979; Raivio *et al.*, 1981), was the apparent lack of a positive correlation between the liver concentrations of P_i and *PPRibP*. Previous reports show that, although comparatively large changes in extracellular P_i (25–60 mM) are required to increase the activity of *PPRibP* synthetase in intact cells (Bashkin & Sperling, 1978), much smaller variations in the P_i content of the medium (1–5 mM) dramatically change the activity of the enzyme when purified preparations of the enzyme are used and no cell barriers have to be circumvented. This discrepancy between the extracellular and intracellular P_i concentrations can largely be explained in terms of the high gradient across the cell membrane for this ion. Planet & Fox (1976) have shown that exposing erythrocytes to an external medium containing 0, 10 mM or 20 mM- P_i resulted in intracellular values of 0.5, 1.5 and 2.4 mM. These latter concentrations are well within the range which Planet & Fox (1976) found as being capable of producing substantial increases in cellular *PPRibP*. The total P_i per g of liver found in the present study is of the order of 2.7–4.1 mM, i.e. within the range where it might be expected that the higher concentrations of intracellular P_i would be associated with the higher concentrations of *PPRibP*. In the present work, the reverse appears to be true, with a negative correlation existing between the P_i and *PPRibP* contents, and this would suggest that alternative forms of regulation must be considered.

One conclusion to be drawn from the present data on freeze-clamped liver preparations from rats under different short-term hormonal or dietary conditions is that ATP and the energy charge of the cell have an over-riding regulatory effect on hepatic *PPRibP* concentration. This does not preclude effects of P_i in particular cell compartments, or an indirect action of P_i , e.g. as manifest through regulation of glycolytic flux, and thus of ATP concentrations, in the cytosol, although the apparent negative correlation between hepatic *PPRibP* concentration and P_i concentrations makes it unlikely that P_i is the dominant regulator of hepatic *PPRibP* *in vivo*. The K_m for ATP for purified human erythrocyte *PPRibP* synthetase has been reported as 14 μ M (Fox &

Fig. 3. Relationship between the hepatic *PPRibP* concentration and the flux of glucose through the pentose phosphate pathway and the contents of pentose-phosphate-pathway intermediates in different dietary and hormonal conditions

(a) Hepatic *PPRibP* concentrations and 6-phosphogluconate (6PG) and xylulose 5-phosphate (Xyl-5-P) contents and the flux through the oxidative pentose phosphate pathway (PPP; C-1–C-6). The values given are means $\pm$ S.E.M. (of not less than six values); the vertical lines represent twice the S.E.M. (b) Hepatic *PPRibP* concentrations and ribose 5-phosphate (Rib-5-P) and the flux through the oxidative pentose phosphate pathway (PPP), plotted on a log scale. Ribose 5-P was calculated from the tissue content of xylulose 5-P by using the equilibrium constants of ribose-5-phosphate isomerase and ribulose-phosphate 3-epimerase as given by Casazza & Veech (1986b).

Kelley, 1971). It seems possible that, at the high cell concentrations of ATP found *in vivo* (of the order of 2–3 mM), the kinetic parameters of other effector molecules on hepatic *PPRibP* synthetase may be modified.

Supply of ribose 5-phosphate

There are a number of systems in which it has been shown that, given the appropriate concentration of P_i , the rate of formation of *PPRibP* is related to the supply of ribose 5-phosphate via the oxidative pentose phosphate pathway. Bashkin & Sperling (1978) have demonstrated that the addition of ascorbate or of Methylene Blue, both stimulators of glucose flux in the pentose phosphate pathway, to cultures of epithelial-like hepatic cells increased the cellular concentration of ribose 5-phosphate and the incorporation of labelled adenine into nucleotides, changes signifying an increased bioavailability of *PPRibP*.

In the present experiments, three parameters linked the

increased flux of glucose through the oxidative pentose phosphate pathway with the tissue 'steady-state' concentration of *PPRibP*, each correlating to a high degree of significance. These were: (i) the flux of glucose through the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reactions, as shown by the yields of $^{14}\text{CO}_2$ from C-1–C-6 of glucose (Fig. 3); (ii) the redox state of $\text{NADP}^+/\text{NADPH}$ and the concentration of 6-phosphogluconate and pentose phosphate in the liver (Fig. 3); and (iii) the activities of the enzymes of the oxidative segment of the pentose phosphate pathway, including ribose-5-phosphate isomerase (Table 2). These lines of evidence, taken together, strongly suggest that *PPRibP* formation *in vivo* is dependent on the increased supply of ribose 5-phosphate arising from the oxidation of glucose 6-phosphate to 6-phosphogluconate and to ribulose 5-phosphate, followed by the conversion into ribose 5-phosphate. It is noteworthy that ribose-5-phosphate isomerase activity paralleled *PPRibP*, whereas that of ribulose-phosphate 3-epimerase, which

Table 2. Correlation between hepatic *PPRibP* concentration and enzymes of the oxidative and non-oxidative routes of pentose phosphate formation in diabetes, with and without insulin treatment, starvation and starvation followed by high-carbohydrate and high-fat diets

Results [units ($\mu\text{mol}/\text{min})/\text{g}$ of liver at 25°C] are given as means $\pm$ s.e.m. of not less than six values in each group. Fisher's *P* values are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. N.S., not significant. The correlation coefficients (*r*) are calculated from the enzyme activities given in this Table and the *PPRibP* values given in Table 1.

Condition	Glucose-6-phosphate dehydrogenase	6-Phosphogluconate dehydrogenase	Ribose-phosphate isomerase	Ribulose-phosphate 3-epimerase	Transketolase	Transaldolase
Control	1.13 $\pm$ 0.08	2.54 $\pm$ 0.22	3.24 $\pm$ 0.12	26.3 $\pm$ 0.6	1.71 $\pm$ 0.06	1.11 $\pm$ 0.06
Diabetic 5 days	0.805 $\pm$ 0.062*	2.68 $\pm$ 0.18	2.90 $\pm$ 0.06	23.3 $\pm$ 0.6	1.54 $\pm$ 0.05	1.13 $\pm$ 0.04
Control	1.17 $\pm$ 0.05	2.81 $\pm$ 0.10	3.51 $\pm$ 0.21	23.5 $\pm$ 1.1	1.74 $\pm$ 0.04	1.08 $\pm$ 0.06
Diabetic 20 days	0.741 $\pm$ 0.056***	1.81 $\pm$ 0.30**	2.87 $\pm$ 0.36	19.4 $\pm$ 1.1*	1.21 $\pm$ 0.07***	1.15 $\pm$ 0.06
Diabetic 20 days + insulin (5 i.u./day) 3 days	2.69 $\pm$ 0.21***	4.53 $\pm$ 0.35***	3.80 $\pm$ 0.50	13.9 $\pm$ 1.0***	2.43 $\pm$ 0.11***	2.62 $\pm$ 0.14***
Control	1.12 $\pm$ 0.14	2.96 $\pm$ 0.07	3.10 $\pm$ 0.08	21.6 $\pm$ 1.0	1.67 $\pm$ 0.08	1.18 $\pm$ 0.03
Starved 3 days	0.895 $\pm$ 0.09	2.44 $\pm$ 0.14**	2.94 $\pm$ 0.13	18.6 $\pm$ 0.8*	2.01 $\pm$ 0.16	1.35 $\pm$ 0.05*
Starved 3 days + high-carbohydrate diet 3 days	3.22 $\pm$ 0.41***	3.65 $\pm$ 0.32	4.17 $\pm$ 0.10***	22.8 $\pm$ 1.0	1.83 $\pm$ 0.07	1.48 $\pm$ 0.04***
Starved 3 days + high-fat diet 3 days	0.96 $\pm$ 0.06	2.57 $\pm$ 0.11*	3.32 $\pm$ 0.10	20.4 $\pm$ 0.9	1.65 $\pm$ 0.08	1.39 $\pm$ 0.04**
<i>r</i>	0.861	0.628	0.788	0.085	0.404	0.261
<i>P</i>	< 0.001	< 0.05	< 0.01	NS	NS	NS

directs pentose phosphate to the non-oxidative segment of the pentose phosphate pathway and is the more active enzyme, did not, nor did the activity of transketolase or transaldolase (Table 2). The importance of ribose phosphate isomerase in directing glucose metabolism in the pentose phosphate pathway towards ribose 5-phosphate formation is supported by the measurement of the hepatic enzyme profile in rats bearing the growth-hormone-secreting pituitary tumour STW6, where liver-weight increases were accompanied by a greater increase in this enzyme than in the other enzymes of the pentose phosphate pathway (Gumaa *et al.*, 1969).

Calculating the hepatic ribose 5-phosphate from the measured xylulose 5-phosphate content, by using the equilibrium constants determined by Casazza & Veech (1986b), it is found that the normal control value is approx. $8\ \mu\text{M}$, compared with $3.5\ \mu\text{M}$ reported by Casazza & Veech (1986a). The similarly calculated range of ribose 5-phosphate concentrations in the experimental groups in the present experiments is $3\text{--}50\ \mu\text{M}$. Fox & Kelley (1971) reported the K_m of the purified erythrocyte enzyme to be $33\ \mu\text{M}$, so that the present values span the important physiological range.

The present results appear to indicate that *PPRibP* bioavailability in liver is most closely controlled by the energy charge and supply of ribose 5-phosphate via the pentose phosphate pathway, and that a lesser role is played by P_i and the absolute amount of *PPRibP* synthetase present.

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