Concentration of phosphoribosyl pyrophosphate in the kidney during development and in experimental diabetic hypertrophy

Sirilaksana KUNJARA,* Milena SOCHOR, Abimbola ADEOYA, Patricia McLEAN and A. Leslie GREENBAUM
Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London W1P 7PN, U.K.

The effect of developmental growth on the kidney content of phosphoribosyl pyrophosphate *PPRibP* was studied in rats at ages between the foetal animal and up to 100 days of age. In addition, the effect of short-term diabetes (up to 14 days) on the renal content of *PPRibP* was studied in immature rats and in adults aged approx. 60 days. The developmental pattern of *PPRibP* is such that the *PPRibP* content is lowest in the young rat and increases as the rate of kidney growth slows. In the adult rat, the early kidney hypertrophy of diabetes is accompanied by a fall in *PPRibP* content and, again, the *PPRibP* content returns to normal as the rate of kidney hypertrophy diminishes. Induction of diabetes in the immature rat causes a lesser degree of kidney hypertrophy and also a smaller depression of renal *PPRibP* content. The activity of *PPRibP* synthetase (EC 2.7.6.1) is not significantly affected by age or diabetes. The changes in *PPRibP* content are discussed in relation to the generation of ribose 5-phosphate by the pentose phosphate pathway and the utilization of *PPRibP* for nucleotide synthesis via the 'de novo' and salvage pathways.

INTRODUCTION

The renal complications of diabetes include a rapid initial hypertrophy in the short term, and severe structural and pathophysiological damage in the longer term. Studies (Seyer-Hansen, 1976, 1983) have shown that there is an early increase in RNA and protein synthesis and a rise in the rate of incorporation of precursors into pyrimidine nucleotides and their derivatives in the kidney during diabetes-induced growth (Cortes *et al.*, 1980*a,b*, 1982). The kidney growth rate has been shown to be positively correlated with the blood glucose concentration, and the initial renal hypertrophy can be reversed by insulin (Seyer-Hansen, 1976, 1977). Studies in our laboratory have shown that a positive correlation exists between the activity of the renal pentose phosphate pathway, which produces ribose 5-phosphate for nucleotide and nucleic acid synthesis, and the growth rate of the kidney in diabetic, unilateral-nephrectomized and diabetic-unilateral-nephrectomized rats (Steer *et al.*, 1982, 1985). The nature of the link between the increased blood glucose and increased activity of the pentose phosphate pathway on the one hand and the increased RNA synthesis and kidney growth on the other remains to be explained. A key intermediate, located at the centre point of these biosynthetic processes, is phosphoribosyl pyrophosphate (*PPRibP*). Although there is some evidence for an increased bioavailability of *PPRibP* in the diabetic rat kidney *in vivo* (Cortes *et al.*, 1980*b*), there is, as yet, little or no direct information on the renal content and the regulation of the metabolism of this important intermediate in diabetes. The present study examined the tissue content of *PPRibP* in alloxan- and streptozotocin-induced diabetes at time intervals up to 14 days after the induction of diabetes. Comparison was made of the

effects of diabetes in immature rats, where the kidneys were already in a phase of rapid growth, with the effects in adult animals, where the kidneys had achieved steady-state growth with respect to body weight. Finally, the developmental pattern of renal *PPRibP* content and kidney growth was also studied from the late-foetal stage up to 100 days of age. A negative correlation was found to exist between the renal content of *PPRibP* and the rate of kidney growth in developing animals and in the initial kidney hypertrophy of adult diabetic animals. In immature animals, the effect of diabetes on both tissue *PPRibP* content and rate of kidney growth was less than in mature rats. The basis of such differences is discussed.

METHODS

Animals

Experimental diabetes. Four groups of rats were used.

(a) Rats were taken at different ages, from late pregnancy to 15 weeks old, for the study of the developmental pattern of change of *PPRibP* content of the kidney. These rats were fed *ad lib.* with free access to water, and were taken at the appointed age to be killed by cervical dislocation, without any treatment.

(b) Group 2 consisted of immature rats aged 20 days and weighing 45 g. These rats were injected intraperitoneally with a freshly prepared solution of streptozotocin (Boehringer, Mannheim, Germany), dissolved in 0.05 M-sodium phosphate buffer, pH 4.0, at a dose of 12 mg/100 g body wt. For calculation of the duration of treatment, the day of injection was taken as zero time. Rats in this group were killed, by cervical dislocation, at 3, 5, or 14 days after the induction of diabetes.

Abbreviation used: *PPRibP*, phosphoribosyl pyrophosphate.

* Permanent address: Department of Biochemistry, Pramongkutklao College of Medicine, Bangkok, Thailand.

(c) Group 3 consisted of adult rats aged 8 weeks and weighing 150–200 g. These rats were injected with streptozotocin (12 mg/100 g body wt, intraperitoneally) as for Group 2, and were killed 3, 5 or 14 days after the induction of diabetes. Additionally, one set of rats, designated 3-days-diabetic, were treated with streptozotocin and then given 2 units (intravenously) of Monotard insulin 6, 24 and 30 h after the streptozotocin in order to maintain, as far as practical, normal blood glucose concentrations. Insulin concentrations were assumed to have diminished by 48 h, when the blood glucose began to increase appreciably. These rats were killed after a further 3 days and the results are given in Table 3 as '3-days-diabetic rats'. This treatment was adopted to minimize any short-term toxic effect of the drug itself, as distinct from the diabetic state produced.

(d) Group 4 consisted of adult rats aged 8 weeks and weighing 200–220 g. These rats were injected intraperitoneally with a solution of alloxan monohydrate (Sigma, Poole, Dorset, U.K.) dissolved in acetate buffer, pH 5.4, and used immediately. The dose was 20 mg/100 g body wt. These rats had been starved for the 24 h before the injection and were fed 8 h after the injection. These rats were killed at either 3, 5 or 14 days after the injection.

Preparation of tissue extracts

Determination of *PPRibP* content. One kidney (or two kidneys for the young animals given in Table 1) was rapidly removed from an animal killed by cervical dislocation, weighed, and dropped into 5 or 10 ml of 100 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA, which had been pre-heated to 100 °C in a boiling-water bath. The choice of volume of homogenizing medium was set by the weight of the kidney so that the extract was between 1:5 and 1:10 (w/v). The kidney was immediately homogenized with an Ultra-Turrax blender (Janke and Kunkel, Staufen i. Breisgau, Germany) for 30 s and returned to the boiling-water bath for a total time of 5 min. The concentration of buffer used here was greater than that used by Reem & Friend (1976) for liver, as was the inactivation time, but these increases were found necessary to allow complete inactivation of the unusually high phosphatase activity of the kidney. Shorter boiling times gave extracts with a lower *PPRibP* content and in which the *PPRibP* content rapidly declined on standing on ice. The heated extracts were chilled in ice for 2 min and then centrifuged in an Eppendorf 3200 centrifuge for 1 min. The supernatant from this was used directly without further treatment.

Determination of *PPRibP* synthetase. Extracts were prepared essentially as described by Lipstein *et al.* (1978), except that a more extended dialysis procedure (2 × 1 h) was used.

Assay procedures

Assay of *PPRibP*. The method used was essentially that described by Buchanan *et al.* (1982), except that carrier orotic acid (0.2 mM) was added and the amount of the orotidine 5'-phosphate pyrophosphorylase/orotidine 5'-phosphate decarboxylase enzyme mixture was increased from 1 to 3 mg per assay tube. The increased amount of enzyme permitted a decrease in the incubation time from 20 to 10 min at (37 °C) and gave better recoveries of standard *PPRibP* additions. The $^{14}\text{CO}_2$ produced by the decarboxylase was collected in Hyamine and counted for

radioactivity in a Beckman model LS7500 scintillation counter.

Assay of *PPRibP* synthetase. The activity of this enzyme (EC 2.7.6.1) was measured by the method of Roth *et al.* (1978), with two modifications. The first of these was the addition of 0.25 mM-dithiothreitol (final concn.) to the assay mixture, and the second was the substitution of a system for the measurement of *PPRibP* based on the conversion of labelled orotic acid into orotidine 5'-phosphate and the decarboxylation of this product by orotidine 5'-phosphate decarboxylase, to yield $^{14}\text{CO}_2$, in place of the published system, which used hypoxanthine/guanosine phosphoribosyltransferase.

The synthetase assay mixture contained 20 mM- P_i , i.e. sufficient to achieve maximal activation of the enzyme (Bashkin & Sperling, 1978; Becker *et al.*, 1979). The assay was carried out in a plastic tube. The incubation mixture for *PPRibP* assay was (final concns.): potassium phosphate, pH 7.6, 60 mM; MgSO_4 , 17 mM; Na_2EDTA , pH 7.6, 0.5 mM; dithiothreitol, 0.3 mM; orotic acid, 0.6 mM; 0.15 μCi of [*carboxy*- ^{14}C]orotic acid (52 Ci/mol) (New England Nuclear) and 3 mg of the mixed enzyme preparation containing orotidine 5'-phosphate pyrophosphorylase and orotidine 5'-phosphate decarboxylase (Sigma), all in a volume of 215 μl . To this mixture was added 20 μl of dialysed extract as prepared above, and the volume was made up to 700 μl with water. After insertion of a collecting tube containing 0.5 ml of 1 M-Hyamine in methanol, the tube was sealed and the whole incubated at 37 °C for 5 min. The reaction was started by the injection of 40 μl of mixture containing 25 mM-ribose 5-phosphate and 25 mM-ATP and was allowed to continue for 15 min. At the end of this time, 0.5 ml of 1 M- H_2SO_4 was injected through the stopper and the collection of $^{14}\text{CO}_2$ continued for a further 30 min. The Hyamine was collected and counted for radioactivity as above. The unit of enzyme activity was defined as that producing 1 μmol of product in 1 h at 37 °C.

Protein determination. The protein content of both the total kidney homogenates and of the supernatant fraction used for *PPRibP* synthetase was estimated by the method of Lowry *et al.* (1951).

RESULTS

PPRibP content of kidneys taken from rats at different stages of development

Table 1 shows the *PPRibP* content of kidneys taken from rats at different stages of development, from the late-foetal stage up to 100 days *post partum*: it is lowest in the foetal kidney and rises with increasing age. The rise is most rapid in the first 20 days of extra-uterine life, at the period of most rapid kidney growth, and slows to reach a virtual plateau value, by 40–50 days *post partum*.

In this latter period, the kidney growth is slower and is more nearly proportional to the increase in body weight. In the earlier phase of development there is a strong correlation between kidney *PPRibP* content and age ($r = 0.802$, $n = 36$, $P < 0.001$) and between kidney *PPRibP* content and kidney weight ($r = 0.76$, $n = 36$, $P < 0.001$). Finally, taken over the whole development period studied, there is a strong negative correlation between the renal *PPRibP* content and the rate of kidney

Table 1. Phosphoribosyl pyrophosphate content of the kidneys taken from rats at different ages during developmentResults are means $\pm$ S.E.M. for the numbers of animals shown.

Age (days)	No. of animals	Body wt. (g)	Kidney wt.* (g)	PPRibP (nmol/g of kidney)
Foetal				
2	4	3.8 $\pm$ 0.10	0.022 $\pm$ 0.002†	2.93 $\pm$ 0.10
Post partum				
1	4	6.9 $\pm$ 0.11	0.09 $\pm$ 0.004	4.01 $\pm$ 0.47
5	4	15.7 $\pm$ 1.9	0.162 $\pm$ 0.005	3.88 $\pm$ 1.04
10	8	21.1 $\pm$ 0.4	0.280 $\pm$ 0.007	4.25 $\pm$ 0.52
15	5	26.6 $\pm$ 1.2	0.463 $\pm$ 0.014	6.29 $\pm$ 0.57
20	12	54.7 $\pm$ 1.2	0.714 $\pm$ 0.017	6.47 $\pm$ 0.27
30	7	89.1 $\pm$ 4.1	0.991 $\pm$ 0.027	8.17 $\pm$ 0.56
40	12	119 $\pm$ 3	1.25 $\pm$ 0.03	8.34 $\pm$ 0.43
55	17	209 $\pm$ 7	1.89 $\pm$ 0.04	9.33 $\pm$ 0.28
65	25	235 $\pm$ 3	2.17 $\pm$ 0.04	9.85 $\pm$ 0.27
80	4	303 $\pm$ 13	2.29 $\pm$ 0.07	8.65 $\pm$ 0.44
90	6	320 $\pm$ 9	2.62 $\pm$ 0.17	8.84 $\pm$ 0.35
100	4	417 $\pm$ 16	3.39 $\pm$ 0.20	10.87 $\pm$ 1.11

*Two kidneys.

†Calculated from weight of total kidney of all foetuses *in vitro* and expressed as content of two kidneys.

growth (as measured by the percentage increase in kidney weight/day) ($r = 0.96$, $n = 11$, $P < 0.001$).

Effect of diabetes and the duration of diabetes on kidney growth and PPRibP content

Mature animals. Both alloxan- and streptozotocin-induced experimental diabetes were studied (Table 2), the diabetes being of 3, 5 or 14 days duration.

In alloxan-diabetic rats, marked kidney hypertrophy was observed at the shorter time intervals, the increase being statistically significant, but the gain was less marked at the longer time. The PPRibP content showed a reciprocal pattern, being significantly decreased at the two shorter time intervals and unchanged at 14 days. A similar pattern was observed in streptozotocin-treated rats, but the changes were less pronounced. The kidney weight was significantly increased only after 5 days of diabetes, although an appreciable, but not significant hypertrophy had occurred at the shorter time interval. Again, kidney growth was associated with decreased PPRibP content, this being significantly lower in the diabetic kidneys than in controls at all stages studied.

In view of the known nephrotoxic effects of diabetogenic agents such as alloxan and streptozotocin (Evan *et al.*, 1984a,b) and the complications of the rapid changes in blood insulin released from the damaged pancreas which follow the administration of these compounds, it was considered desirable to discount, as far as possible, these early transient changes by maintaining streptozotocin-treated rats on insulin over the initial period and then timing the onset of diabetes as the time of withdrawal of insulin 48 h after injection of streptozotocin. This approach has been usefully employed by Seyer-Hansen (1976).

The results of such an experiment are given in Table 3, showing that both the kidney growth and the fall of PPRibP content, shown in Table 2, are still observed, and it appears therefore that these changes must be related to the diabetogenic, rather than the toxic, effect of the drug.

(ii) Immature animals. Only streptozotocin-diabetes was studied in this group of animals. The data in Table 4 show that diabetes had little effect on kidney growth when crude kidney weight is considered, but, when the growth is expressed as kidney wt./100 g body wt., the kidneys of the diabetic rats were significantly larger than those of the age-related controls at each of the times studied. In contrast with the adult animals, the kidney growth was not associated with any fall in PPRibP content except in the 14-day-diabetic group. This exception may be related to the fact that, at death, this last group were aged 34 days and, quite clearly, on the basis of the data in Table 1, should be considered mature. The lower PPRibP content of the kidney of diabetic rats at this time reflects not so much a fall in PPRibP, as shown to occur in the kidney of diabetic animals (Table 2), but an age-related rise in PPRibP in the control animals, as shown in Table 1.

Activity of PPRibP synthetase in the kidneys of normal and diabetic rats.

Table 5 shows the effect of diabetes on the activity of PPRibP synthetase in the kidneys of adult and immature animals. The duration of diabetes in these experiments was chosen to match times at which large changes in the PPRibP content had been observed (Tables 2 and 4). The results have been calculated on a wet-weight basis and also as activity/mg of protein to allow for changes of water content under the different conditions. The activity of the enzyme was essentially the same in young and adult rats and was unchanged by diabetes under any of the conditions tested.

DISCUSSION

The kidney appears to be exceptional among the tissues of the adult diabetic rat (Pain & Garlick, 1974) in that it shows a marked growth, characterized by both hypertrophy and hyperplasia, in the early stages of diabetes, in contrast with the general pattern of tissue

Table 2. Effect of experimental diabetes on the growth and phosphoribosyl pyrophosphate content of the kidneys of mature ratsResults are given as means $\pm$ S.E.M. Fisher's *P* values are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001

Condition	No. of animals	Initial body wt. (g)	Final body wt. (g)	Kidney wt.† (g)	Kidney wt./100 g body wt. (g)	PPRibP (nmol/g)	Blood glucose (mm)
(a) Alloxan-diabetic							
Control (C)	9	200–220	229 $\pm$ 2	2.03 $\pm$ 0.04	0.87 $\pm$ 0.04	9.90 $\pm$ 0.041	7.7 $\pm$ 0.6
3 days diabetic (D)	11		181 $\pm$ 4	2.75 $\pm$ 0.10	1.52 $\pm$ 0.11	6.14 $\pm$ 0.41	42.8 $\pm$ 3.7
D/C (%)			79***	136***	171***	62***	560***
Control (C)	8	200–220	234 $\pm$ 5	2.03 $\pm$ 0.05	0.87 $\pm$ 0.08	10.57 $\pm$ 0.34	6.9 $\pm$ 0.04
5 days diabetic (D)	13		191 $\pm$ 6	2.80 $\pm$ 0.05	1.47 $\pm$ 0.11	7.78 $\pm$ 0.58	32.9 $\pm$ 1.7
D/C (%)			81***	138***	169***	74***	480***
Control (C)	4	200–220	250 $\pm$ 4	2.29 $\pm$ 0.16	0.92 $\pm$ 0.10	9.16 $\pm$ 0.50	6.5 $\pm$ 0.5
14 days diabetic (D)	5		212 $\pm$ 12	2.55 $\pm$ 0.17	1.20 $\pm$ 0.13	8.62 $\pm$ 0.96	26.5 $\pm$ 3.0
D/C (%)			85*	111	130	94	408***
(b) Streptozotocin-diabetic							
Control (C)	4	160–180	187 $\pm$ 20	1.70 $\pm$ 0.13	0.91 $\pm$ 0.07	9.09 $\pm$ 0.06	7.5 $\pm$ 0.5
3 days diabetic (D)	4		158 $\pm$ 4	1.95 $\pm$ 0.07	1.23 $\pm$ 0.06	5.67 $\pm$ 0.50	20.0 $\pm$ 1.4
D/C (%)			84	115	135*	62**	267***
Control (C)	4	160–180	181 $\pm$ 5	1.66 $\pm$ 0.02	0.92 $\pm$ 0.08	9.48 $\pm$ 0.42	6.0 $\pm$ 1.2
5 days diabetic (D)	7		169 $\pm$ 5	2.12 $\pm$ 0.06	1.25 $\pm$ 0.08	7.42 $\pm$ 0.60	27.4 $\pm$ 0.09
D/C (%)			94	127***	136*	78*	457***
Control (C)	4	180–200	245 $\pm$ 6	2.06 $\pm$ 0.04	0.84 $\pm$ 0.06	9.24 $\pm$ 0.20	6.5 $\pm$ 1.0
14 days diabetic (D)	8		169 $\pm$ 8	2.22 $\pm$ 0.08	1.39 $\pm$ 0.12	7.95 $\pm$ 0.39	21.6 $\pm$ 1.5
D/C (%)			69***	108	165***	86*	332***

†Weight of two kidneys.

Table 3. Effect of streptozotocin-diabetes on the kidney content of phosphoribosyl pyrophosphate in rats which had been protected from the short-term toxic effects of the drug by treatment with insulin

The experimental rats were rendered diabetic by the injection of streptozotocin (12 mg/100 g body wt., intraperitoneally) and then maintained on insulin (Monotard, 2 units at 6, 24 and 30 h after induction of diabetes). The onset of diabetes was taken as being 48 h after injection of streptozotocin (see the Methods section). Results are given as means $\pm$ S.E.M. Fisher's *P* values are shown by ***P* < 0.01; ****P* < 0.001.

Condition	No. of animals	Initial body wt. (g)	Final body wt. (g)	Kidney wt.† (g)	Kidney wt./100 g body wt. (g)	PPRibP content (nmol/g)	Blood glucose (mm)
Control (C)	4	180–190	196 $\pm$ 1.8	1.77 $\pm$ 0.05	0.904 $\pm$ 0.020	8.56 $\pm$ 0.07	7.9 $\pm$ 0.8
3 days diabetic (D)	4	180–190	170 $\pm$ 3.9	2.13 $\pm$ 0.08	1.256 $\pm$ 0.018	6.65 $\pm$ 0.44	27.8 $\pm$ 2.6
D/C (%)			87**	120**	139***	78**	352***

†Weight of two kidneys.

loss. The studies by Seyer-Hansen (1977) and by Steer *et al.* (1982, 1985) link this growth with the high blood glucose and increased activity of the pentose phosphate pathway, both of which originate in the raised intracellular concentration of glucose, consequent on the free permeability of the kidney to this substrate in the absence of insulin.

The increased RNA content of the kidney in early experimental diabetes could arise from an acceleration of RNA formation (Cortes *et al.*, 1980a) or a decrease in RNA breakdown. This latter possibility, proposed by Hill *et al.* (1974) to account for the renal hypertrophy following unilateral nephrectomy, cannot be ruled out in the renal hypertrophy of diabetes, particularly in view of

the report by Cortes *et al.* (1980a) that insulin promotes a rapid decline in the RNA content of the diabetic rat kidney. However, evidence is available to show that increased purine synthesis *de novo* also plays some part (Cortes *et al.*, 1980a), with the further possibility of an increased incorporation of the products of the salvage pathway also being involved. Both of these latter routes would require the presence of PPRibP and, indeed, Cortes *et al.* (1980a) have provided evidence that the 'bioavailability' of PPRibP may constitute a limiting factor in such incorporations. It is this requirement for PPRibP which may link the observed correlation of a high blood glucose and of PPRibP concentration to the growth process.

Table 4. Effect of streptozotocin-diabetes on the growth and phosphoribosyl pyrophosphate content of the kidneys of immature ratsResults are given as means $\pm$ S.E.M. Fisher's *P* values are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Condition	No. of animals	Initial body wt. (g)	Final body wt. (g)	Kidney wt.† (g)	Kidney wt./100 g body wt. (g)	PPRibP content (nmol/g)	Blood glucose (mM)
Control (C)	5	45–50	53.5 $\pm$ 2.0	0.672 $\pm$ 0.019	1.26 $\pm$ 0.03	5.85 $\pm$ 0.40	5.9 $\pm$ 0.4
3 days diabetic (D)	6		41.3 $\pm$ 1.1	0.722 $\pm$ 0.018	1.75 $\pm$ 0.06	4.82 $\pm$ 1.06	19.0 $\pm$ 1.8
D/C (%)			77***	107	137***	82	322***
Control (C)	6	45–50	51.6 $\pm$ 1.5	0.672 $\pm$ 0.034	1.30 $\pm$ 0.05	4.94 $\pm$ 0.33	6.5 $\pm$ 0.4
5 days diabetic (D)	6		45.7 $\pm$ 1.5	0.781 $\pm$ 0.014	1.71 $\pm$ 0.05	4.41 $\pm$ 0.18	21.4 $\pm$ 1.5
D/C (%)			88**	116*	131***	89	329***
Control (C)	6	45–50	93.6 $\pm$ 4.2	0.896 $\pm$ 0.039	0.96 $\pm$ 0.03	8.86 $\pm$ 0.49	6.3 $\pm$ 0.3
14 days diabetic (D)	6		73.4 $\pm$ 4.9	1.101 $\pm$ 0.061	1.50 $\pm$ 0.04	5.97 $\pm$ 0.16	20.9 $\pm$ 0.7
D/C (%)			78**	123*	157***	67***	332***

†Weight of two kidneys

Table 5. Activity of phosphoribosyl pyrophosphate synthetase in the kidneys of diabetic ratsResults are given as means $\pm$ S.E.M. Fisher's *P* values are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Condition	No. of animals	Initial body wt. (g)	Final body wt. (g)	Kidney wt. (g)	PPRibP synthetase activity	
					(μ mol/h per g of tissue at 37 °C)	(μ mol/h per mg of protein at 37 °C)
Adult rats						
Control (C)	6	200	231 $\pm$ 2.7	2.073 $\pm$ 0.050	26.2 $\pm$ 1.2	0.355 $\pm$ 0.016
3 days alloxan-diabetic (D)	6	200	181 $\pm$ 7.6	2.779 $\pm$ 0.089	23.8 $\pm$ 11	0.321 $\pm$ 0.009
D/C (%)			78***	134***	91	90
Control (C)	6	200	233 $\pm$ 6.4	2.049 $\pm$ 0.035	24.6 $\pm$ 0.4	0.345 $\pm$ 0.006
5 days alloxan-diabetic (D)	6	200	196 $\pm$ 9.3	2.623 $\pm$ 0.086	24.8 $\pm$ 0.8	0.352 $\pm$ 0.011
D/C (%)			84**	128**	101	102
Immature rats						
Control (C)	6	50	110 $\pm$ 4.1	1.202 $\pm$ 0.052	23.6 $\pm$ 0.2	0.347 $\pm$ 0.019
14 days streptozotocin-diabetic	5	50	88 $\pm$ 5.4	1.311 $\pm$ 0.058	23.2 $\pm$ 0.2	0.335 $\pm$ 0.022
D/C (%)			80**	109*	98	96

The sequence of reactions linking glucose to nucleotide synthesis includes the following steps.

(i) Conversion of glucose into glucose 6-phosphate; this is increased, as shown by (a) an increased tissue content of glucose 6-phosphate (Needleman *et al.*, 1968; Steer *et al.*, 1985) and (b) an increased activity of hexokinase (Sochor *et al.*, 1979, 1985).

(ii) Flux through the pentose phosphate pathway; this is increased in diabetes, as shown by (a) measurements of glucose-6-phosphate dehydrogenase and of 6-phosphogluconate dehydrogenase (Steer *et al.*, 1982, 1985) and (b) measurements of the metabolism of differentially labelled glucose (Sochor *et al.*, 1979; Steer *et al.*, 1982, 1985).

(iii) Formation of ribose 5-phosphate; this is probably increased in diabetes, as shown by the increased activity of the oxidative, and the decreased activity of the non-oxidative, enzymes of the pentose phosphate pathway (Steer *et al.*, 1985) and because the activity of

ribose-5-phosphate isomerase in kidney considerably exceeds that of the oxidative enzymes (Sochor *et al.*, 1986).

(iv) Bioavailability of PPRibP; this is increased in diabetes, as shown by the competition studies of Cortes *et al.* (1980a) for the available PPRibP by orotate on the one hand and the nucleotide bases on the other. There is also an increased orotate incorporation into UTP and RNA in diabetes (Cortes *et al.*, 1980a).

Both the oxidative and the non-oxidative segments of the pentose phosphate pathway can contribute to pentose phosphate formation, required for PPRibP and nucleotide synthesis (Hiatt, 1957, 1958; Gumaa & McLean, 1969a,b; Boss & Pilz, 1985). As emphasized by Horecker (1969), the pentose phosphate pathway can be regarded as two independent routes to ribose 5-phosphate formation, and the relative contributions of each pathway will be heavily dependent, in any one tissue, on factors

stimulating the oxidative route. This is perhaps most clearly seen in ascites cells, where the oxidative route can be stimulated manyfold by phenazine methosulphate, and this segment then becomes the major source of ribose 5-phosphate. In the absence of such stimulation, or in the presence of inhibitors of NADPH oxidation, the non-oxidative sequence is predominant (Gumaa & McLean, 1969*a,b*). In the present experiments, the increase in oxidative-pathway contribution is indicated by the rises of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities and in $^{14}\text{CO}_2$ yield, and the fall in transketolase activity. Indeed, a negative correlation has been found between the rate of growth of the kidney and transketolase activity in early renal hypertrophy in diabetes (Steer *et al.*, 1985), indicative of the predominance of the oxidative route in this additional growth.

In the present experiments, a clear-cut pattern has emerged in which kidney growth, whether normal, developmental or the induced growth following the induction of diabetes, is associated with a lowering of the renal *PPRibP* concentration. In the last case, the possibility that the decrease in *PPRibP* content could be due to the toxic effect of the diabetogenic agent has been eliminated by the experiment (Table 2) in which the onset of the diabetes was delayed by insulin until the toxic effects of alloxan had abated (i.e. 48 h) and was then allowed to proceed by withholding the administered insulin. In these circumstances the kidney growth and the decrease in *PPRibP* were still obtained. Further, as shown by Steer *et al.* (1985), the prevention of the diabetogenic effect of alloxan, by previous injection of glucose, while leaving any possible toxic effect of the drug, prevented the rise in activity of the pentose-phosphate-pathway dehydrogenases which normally accompanies onset of diabetes, again linking the enzyme changes to the diabetogenic, as opposed to the toxic, effect of alloxan. It is therefore not unreasonable to associate the decrease in *PPRibP* with growth, normal or pathological, and not to a drug-induced effect. Nor is this decrease likely to be due to changes in the activation of *PPRibP* synthetase by P_i , probably the most important determinant of activity (Becker *et al.*, 1979; Raivio *et al.*, 1981), since measurements of the P_i content of the kidney show this to be unchanged after 5 days of diabetes [immature rats; controls, 1.23 ± 0.13 ; diabetic, 1.16 ± 0.15 ; adult rats, controls, 1.30 ± 0.017 ; diabetic, 1.22 ± 0.15 ($\mu\text{mol/g}$)].

The earlier findings (see above) that diabetes increases the availability of precursors for *PPRibP* formation together with the present findings that the activity *PPRibP* synthetase is not modified by diabetes (Table 5) and the fall in *PPRibP* concentration (Tables 2–4) all point to the probability that the major determinant in setting the *PPRibP* concentration in the diabetic kidney lies in the steps linking *PPRibP* with the increased nucleic acid synthesis accompanying renal hypertrophy in diabetes, although there is the possibility that the activity of *PPRibP* synthetase is modified. The synthesis of *PPRibP* via the synthetase is regulated by a large number of factors, including product inhibition, energy charge and the concentration of uridine nucleotides (see Becker *et al.*, 1979), all of which may be altered to some degree in the diabetic kidney. It is not therefore possible to state at present whether the fall in *PPRibP* content is related to a decreased rate of synthesis or an increased rate of utilization, or whether both factors contribute.

In this context, it is perhaps relevant that a parallel may be drawn between the present results and the increase in *PPRibP* found in several tissues in conditions where growth is inhibited. For instance, Buesa-Perez *et al.* (1980) have found that inhibition of growth of a mouse leukaemia cell line by methotrexate, an inhibitor of nucleic acid synthesis, increased the *PPRibP* content of the cells some 10-fold, and Tatibana *et al.* (1982) showed that 6-azauridine, which blocks the conversion of orotidylate into UMP, also increased the cellular *PPRibP* in Ehrlich ascites cells and spleen cells. Conversely, Peters *et al.* (1984) have reported that accelerating nucleic acid synthesis by administration of inosine to cultures of a tumour cell line decreases the content of *PPRibP*.

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REFERENCES

- Bashkin, P. & Sperling, O. (1978) *Biochim. Biophys. Acta* **538**, 505–511
- Becker, M. A., Raivio, K. O. & Seegmiller, J. E. (1979) *Adv. Enzymol.* **49**, 281–306
- Boss, G. R. & Pilz, R. B. (1985) *J. Biol. Chem.* **260**, 6054–6059
- Buchanan, J. M., Smith, M. L. & Smith, R. J. (1982) *Adv. Enzyme Regul.* **20**, 135–152
- Buesa-Perez, J. M., Leyva, A. & Pinedo, H. M. (1980) *Cancer Res.* **40**, 139–144
- Cortes, P., Verghese, C. P., Venkatachalam, K. K., Schoenberger, A. M. & Levin, N. W. (1980*a*) *Am. J. Physiol.* **238**, 341–348
- Cortes, P., Levin, N. W., Dumler, F., Rubenstein, A. H., Verghese, C. P. & Venkatachalam, K. K. (1980*b*) *Am. J. Physiol.* **238**, 349–357
- Cortes, P., Dumler, F., Sastry, K. S. S., Verghese, C. P. & Levin, N. W. (1982) *Kidney Int.* **21**, 676–682
- Evan, A. P., Mong, S. A., Connors, B. A., Aronoff, G. R. & Luft, F. C. (1984*a*) *Anat. Res.* **208**, 33–47
- Evan, A. P., Mong, S. A., Gattone, Y. H., Connors, B. A., Aronoff, G. R. & Luft, F. C. (1984*b*) *Renal Physiol.* **7**, 78–89
- Gumaa, K. A. & McLean, P. (1969*a*) *Biochem. Biophys. Res. Commun.* **35**, 86–93
- Gumaa, K. A. & McLean, P. (1969*b*) *Biochem. J.* **115**, 1009–1029
- Hiatt, H. (1957) *J. Biol. Chem.* **229**, 725–732
- Hiatt, H. (1958) *J. Clin. Invest.* **37**, 1461–1464
- Hill, R. M., Ab., G. & Malt, R. A. (1974) *Biochem. J.* **144**, 447–453
- Horecker, B. L. (1969) in *International Symposium on Metabolism, Physiology and Clinical Use of Pentose and Pentitols* (Horecker, B. L., Lang, K. & Takagi, Y., eds.), pp. 5–25, Springer Verlag, Berlin
- Lipstein, B., Boer, P. & Sperling, O. (1978) *Biochim. Biophys. Acta* **521**, 45–54
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Needleman, P., Passonneau, J. V. & Lowry, O. H. (1968) *Am. J. Physiol.* **215**, 655–659
- Pain, V. M. & Garlick, P. J. (1974) *J. Biol. Chem.* **249**, 4510–4514
- Peters, G. L., Laurensse, E., Leyva, A. & Pinedo, H. M. (1984) *FEBS Lett.* **170**, 277–280
- Raivio, K. A., Lazar, C. S. & Becker, M. A. (1981) *Biochim. Biophys. Acta* **678**, 58–64
- Reem, G. & Friend, C. (1976) *J. Cell Physiol.* **88**, 193–196
- Roth, R. G., White, C. & Deuel, T. F. (1978) *Methods Enzymol.* **51**, 12–17

- Seyer-Hansen, K. (1976) *Clin. Sci. Mol. Med.* **51**, 551–555
- Seyer-Hansen, K. (1977) *Diabetologia* **13**, 141–143
- Seyer-Hansen, K. (1983) *Kidney Int.* **23**, 643–646
- Sochor, M., Baquer, N. Z. & McLean, P. (1979) *Arch. Biochem. Biophys.* **198**, 632–646
- Sochor, M., Baquer, N. Z. & McLean, P. (1985) *Mol. Physiol.* **7**, 51–68
- Sochor, M., Kunjara, S., Greenbaum, A. L. & McLean, P. (1986) *Biochem. J.* **234**, 573–577
- Steer, K. A., Sochor, M., Gonzalez, A. M. & McLean, P. (1982) *FEBS Lett.* **150**, 494–498
- Steer, K. A., Sochor, M. & McLean, P. (1985) *Diabetes* **34**, 485–490
- Tatibana, M., Kita, K., Asai, T. & Ikeda, F. (1982) *Eur. J. Biochem.* **128**, 631–636

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