

Renal Hypertrophy in Experimental Diabetes

Changes in Pentose Phosphate Pathway Activity

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SUMMARY

An examination was made of the effect of different periods of experimental diabetes on the activity of the pentose phosphate pathway in rat kidney. A rapid increase in kidney weight, expressed both in absolute terms and in terms of body weight, occurred shortly after the induction of diabetes. The activity of the enzymes of the oxidative segment of the pentose phosphate pathway and the flux of glucose through the pathway were both increased during the first 7 days after induction of diabetes. Thereafter, enzyme activity returned toward control levels, but the increased functional activity of the pathway, as measured using specifically labeled glucose, persisted. In contrast, transketolase was significantly depressed at the time of most rapid kidney growth. A positive correlation was found between the rate of kidney growth and the change in activity of glucose-6-phosphate dehydrogenase and a negative correlation with changes in transketolase activity. The possible roles of the oxidative and nonoxidative segments of the pentose phosphate pathway in the kidney in early diabetes-induced renal hypertrophy are discussed. *DIABETES* 1985; 34:485-90.

The occurrence of a diabetic renal hypertrophy is well established. Shortly after the induction of experimental diabetes in rats, there is a marked increase in kidney weight after only a very short period of hyperglycemia and glycosuria.¹ This rapid phase of kidney growth involves an admixture of cellular hypertrophy and hyperplasia.²

We have recently demonstrated a close and highly significant relationship between the activity of the pentose phosphate pathway and the extent of the renal hypertrophy that has occurred 6 wk after the induction of experimental dia-

betes and/or unilateral nephrectomy.³ The increased functional activity of the pathway may relate to an increased requirement for ribose-5-phosphate, utilized in nucleotide synthesis, and for NADPH, required for reductive biosyntheses. It may also relate to alterations in acid-base and electrolyte balance that are known to influence the activity of the renal pentose phosphate pathway.^{4,5}

In view of the enhanced RNA and DNA synthesis in the kidney in early experimental diabetes, it is reasonable to suppose that there will be an increased demand for nucleotide precursors (ribose-5-phosphate) and reducing power (NADPH) for biosynthetic reactions during this growth period. We have, therefore, investigated the activity of the renal pentose phosphate pathway at timed intervals during the first 6 wk after induction of experimental diabetes in rats. Plasma sodium and potassium were also measured at these times because of the known effects of hyponatremia and hyperkalemia on the activity of this pathway in the kidney.⁴

MATERIALS AND METHODS

Materials. Substrates, coenzymes, and enzymes used in the assay procedures were purchased from Boehringer Corporation Ltd. (London, United Kingdom) or Sigma London (Poole, United Kingdom). Radioisotopes used were obtained from Amersham International.

Animals. Male albino rats of the Wistar strain weighing 200-250 g were used. Diabetes was induced by intraperitoneal (i.p.) injection of alloxan monohydrate (200 mg/kg body wt) into rats previously fasted for 18 h; stock cube diet and water were then allowed ad libitum. The rats were killed 2, 7, 21, and 42 days after the induction of diabetes.

The possible nephrotoxic effects of alloxan were evaluated by injection of the same diabetogenic dose of alloxan into rats that were not fasted and that, in addition, received a glucose load (250 mg glucose injected i.p.) 30 min before injection of alloxan: the blood glucose of these rats remained close to normal limits at 7.1 ± 0.5 mmol/L. A group of alloxan-diabetic rats receiving intensive insulin therapy was also included as a second means of excluding nephrotoxic effects of alloxan as the causative agent in renal hypertrophy and

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an enhanced pentose phosphate pathway activity in diabetes. After administration of alloxan (200 mg/kg body wt) to rats fasted for 18 h, food and water were allowed ad libitum and intensive insulin therapy was commenced as soon as the blood glucose started to rise. This group of rats received four daily injections, at 6-h intervals, of Actrapid MC insulin (4–8 U/day) and Monotard MC insulin (4 U/day) (Novo, Denmark), a regimen designed to maintain blood glucose levels at or below 10 mmol/L over the 2-day period. The dose of Actrapid insulin was adjusted according to the blood glucose at the time of treatment, the blood glucose being monitored by use of the Glucochek 2 reflectance meter before each injection.

A group of rats made diabetic by intravenous (i.v.) injection of streptozocin (STZ, 60 mg/kg body wt) to rats previously fasted for 18 h was also studied after 48 h of diabetes.

The control groups of rats were matched for age and weight with the diabetic groups. The control rats were also fasted for 18 h and received equivalent injections of saline.

The diabetic status at the time of killing the rats was assessed by enzymic spectrophotometric determination of the blood glucose using hexokinase and glucose-6-phosphate dehydrogenase.⁶ Plasma sodium and potassium were estimated by an ion-specific spectra technique.

Enzyme and flux estimations. The activity of the pentose phosphate pathway (PPP) enzymes, glucose-6-phosphate (G6P) dehydrogenase (EC 1.1.1.49), 6-phosphogluconate (6PG) dehydrogenase (EC 1.1.1.44), transketolase (EC 2.2.1.1.), and transaldolase (EC 2.2.1.2) was estimated according to the methods described by Glock and McLean⁷ and Novello and McLean⁸ in dialysed high-speed supernatant fractions of kidney and liver (100,000 rpm × 40 min) as described by Sochor et al.⁹ For all enzyme assays, the reduction of NAD⁺ or NADP⁺ or oxidation of NADH was measured in a Unicam SP8000 recording spectrophotometer. A unit of enzyme activity is defined as 1 μmol of product formed per minute at 25°C.

The flux of glucose through the pentose PPP was measured

by the conversion of a specifically labeled glucose to ¹⁴CO₂ (i.e., difference ¹⁴CO₂ from [1-¹⁴C]glucose and ¹⁴CO₂ from [6-¹⁴C]glucose) by kidney cortex slices incubated for 1 h in Krebs-Ringer bicarbonate medium containing 5 mM glucose (control) and 20 mM glucose (diabetic), and 0.5 μCi [¹⁴C]glucose as previously described.⁹

Metabolite content. The metabolite content of rat kidney was measured using neutralized perchloric acid extracts of rapidly freeze-clamped kidney by standard spectrophotometric procedures given in Bergmeyer.¹⁰ The results are expressed as means ± SEM for not less than six observations. Student's *t*-test was used for statistical analysis of significance.

RESULTS

Pentose phosphate pathway (PPP) activity and duration of diabetes. The effect of different periods of alloxan diabetes in rats on the plasma glucose, Na⁺ and K⁺, body and kidney weight, activity of the pentose phosphate pathway enzymes, and flux of glucose through the PPP in kidney slices is shown in Table 1.

The total kidney weight was significantly increased after only 48 h after alloxan injection. Expressed in terms of body weight, the kidney weight increased to 50% above that of the control at this time interval, but thereafter the changes were less pronounced; the kidney weight/100 g body wt increased only a further 28% after 42 days of alloxan diabetes.

The results in Table 1 show a marked increase in both G6P dehydrogenase and 6PG dehydrogenase activity shortly after the induction of diabetes. These increases correspond to changes in two important parameters: (1) the highly significant lowering of plasma sodium; this observation is in line with the known effect of sodium depletion on the activity of these enzymes;⁴ and (2) the very marked growth of the kidney. The flux of glucose through the PPP, as shown by the difference in ¹⁴CO₂ yields from [1-¹⁴C]glucose and [6-¹⁴C]glucose (C₁ - C₆), was increased approximately fourfold

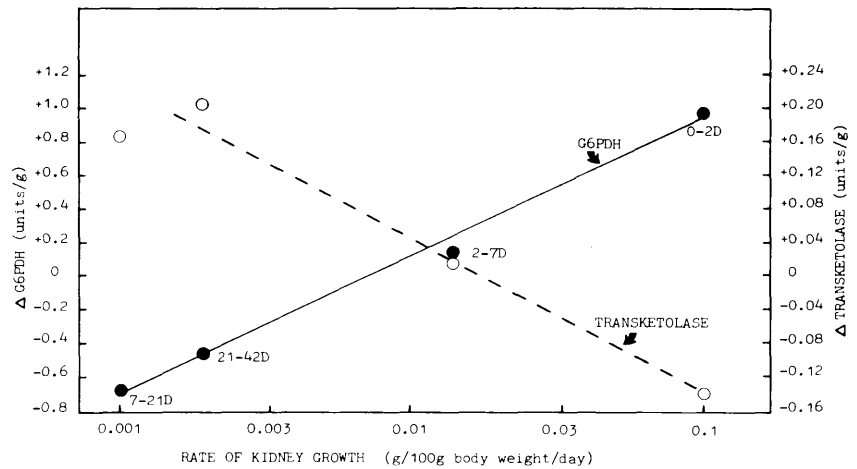
TABLE 1

The effect of different periods of alloxan diabetes on the body and kidney weight, plasma glucose, sodium, potassium, and activity of the pentose phosphate pathway enzymes and flux of glucose through the pathway in rat kidney cortex

	Control	Alloxan diabetic (2 days)	AD2/C (%)	Alloxan diabetic (7 days)	AD7/C (%)	Alloxan diabetic (21 days)	AD21/C (%)	Alloxan diabetic (42 days)	AD42/C (%)
Body wt (g)	288 ± 9	254 ± 11	88*	219 ± 12	76‡	241 ± 11	84‡	257 ± 14	89
Kidney wt (g)	2.28 ± 0.06	3.00 ± 0.12	132‡	2.90 ± 0.16	127‡	3.22 ± 0.14	141‡	3.62 ± 0.20	159‡
Kidney wt/100 g body wt	0.796 ± 0.012	1.19 ± 0.04	150‡	1.33 ± 0.07	167‡	1.34 ± 0.04	168‡	1.41 ± 0.06	178‡
Plasma constituents									
Glucose (mmol/L)	4.90 ± 0.17	47.3 ± 4.30	965‡	33.0 ± 2.31	673‡	29.6 ± 2.45	604‡	28.6 ± 3.90	583‡
Sodium (mmol/L)	142 ± 0.47	128 ± 3.00	90‡	133 ± 1.21	94‡	139 ± 2.05	98	142 ± 0.56	100
Potassium (mmol/L)	3.80 ± 0.10	4.50 ± 0.18	118*	4.18 ± 0.05	110*	4.00 ± 0.12	105	3.70 ± 0.10	97
PPP enzyme activities (U/g)									
G6P-dehydrogenase	0.86 ± 0.03	1.84 ± 0.12	214‡	2.00 ± 0.09	232‡	1.35 ± 0.13	157‡	0.92 ± 0.02	107*
6PG-dehydrogenase	0.92 ± 0.03	1.88 ± 0.14	204‡	2.09 ± 0.25	227‡	0.96 ± 0.04	104	1.07 ± 0.03	116‡
Transketolase	0.93 ± 0.02	0.79 ± 0.04	85‡	0.81 ± 0.07	87	0.98 ± 0.04	105	1.19 ± 0.04	128‡
Transaldolase	1.01 ± 0.83	0.99 ± 0.07	98	1.09 ± 0.05	108	1.08 ± 0.03	107	1.14 ± 0.04	113*
Conversion of glucose to ¹⁴ CO ₂ (μmol/g wet wt/h)									
[1- ¹⁴ C]glucose	3.72 ± 0.08	3.39 ± 0.40	91	3.49 ± 0.36	94	3.54 ± 0.41	81	3.25 ± 0.38	87
[6- ¹⁴ C]glucose	3.47 ± 0.13	2.48 ± 0.19	71‡	2.45 ± 0.19	71‡	2.77 ± 0.27	80*	2.39 ± 0.40	67*
C ₁ - C ₆ (flux via PPP)	0.25 ± 0.11	0.91 ± 0.06	364‡	1.04 ± 0.08	416‡	0.77 ± 0.10	308‡	0.86 ± 0.05	344‡

Each value is the mean ± SEM of not less than six observations. The effect of diabetes on the various parameters is shown as a percentage of the normal control group. Fisher's *P*-values are shown by: **P* < 0.05, †*P* < 0.01, and ‡*P* < 0.001.

FIGURE 1. The correlations between the rate of kidney growth and changes in the activities of G6P dehydrogenase and transketolase are calculated from the data in Table 1. The rate of kidney growth is based on kidney wt/100 g body wt. The incremental changes in G6P dehydrogenase (G6PDH, ●—●) and transketolase (TK, ○—○) are the difference between the enzyme activities measured in control and diabetic groups 0–2 days, 2–7 days, 7–21 days, and 21–42 days, and these points are so indicated on the graph. The correlation coefficient for rate of kidney growth versus change in G6P dehydrogenase is $r = 0.93$, $P < 0.01$, and for rate of kidney growth versus change in transketolase, $r = -0.91$, $P < 0.05$.



at 2 and 7 days after alloxan treatment. Between 21 and 42 days after alloxan treatment, the dehydrogenases of the PPP revert toward the control value. It may be noted that the flux of glucose through the oxidative reactions of the PPP remained 3–4-fold higher than control values at all stages of diabetes (Table 1).

In contrast to the oxidative enzymes of the PPP, the non-oxidative enzymes transketolase and transaldolase show a completely different pattern of response to diabetes. During the most rapid phase of kidney growth, 2 days after alloxan treatment, transketolase activity was significantly decreased, while at the later stages (42 days) activity of the enzyme was significantly increased above control values (Table 1). Transaldolase remained substantially constant throughout the whole period, showing a small (+13%) rise at 42 days.

As shown in Figure 1, there is a positive correlation between the rate of kidney growth at different time intervals and the direction and magnitude of change of G6P dehydrogenase activity ($r = 0.93$, $P < 0.01$); a negative correlation was observed with transketolase activity ($r = -0.91$, $P < 0.05$).

Are the elevated enzyme activities due to a toxic action of alloxan?

To investigate whether the early changes of enzyme activity could be ascribed to short-term toxic effects of alloxan itself, two further groups of animals were studied. In the first group, alloxan was injected but its diabetogenic action was nullified by previous treatment of the rats with a glucose load. In the second group, alloxan action was not prevented but the resultant elevation of the blood glucose was controlled by the concurrent administration of insulin. Enzyme activities were examined 2 days after the injection of alloxan. The results of these experiments are shown in Table 2. In both of these groups, the blood glucose was only slightly elevated and remained well below that observed in the alloxan-treated diabetic groups (Tables 1 and 2). The activities of G6P dehydrogenase and of 6PG dehydrogenase were also less than those observed in the full diabetic groups and were not significantly raised above the control value. Thus, the raised activities of G6P dehydrogenase and of 6PG dehydrogenase reported in Table 1 cannot be ascribed to a direct action of alloxan. Table 2 also shows the effect of STZ-

TABLE 2

Changes of blood glucose, kidney weight, and pentose phosphate pathway enzymes of the kidney cortex 2 days after (1) treatment of starved rats with alloxan (AD), (2) treatment of glucose-loaded rats with alloxan (AND), (3) treatment of alloxan-diabetic rats with insulin injected ab initio (AD+I), and (4) treatment of starved rats with streptozocin (SD)

	Alloxan diabetic (AD)	Glucose-alloxan-treated Nondiabetic (AND)	AND/AD (%)	Alloxan diabetic + insulin (AD+I)	AD+I/AD (%)	Streptozocin diabetic (SD)	SD/C (%)
Body wt (g)	236 ± 10	254 ± 3	107	217 ± 4	92	223 ± 5	77‡
Kidney wt (g)	2.88 ± 0.10	2.18 ± 0.08	76‡	2.06 ± 0.10	71‡	2.26 ± 0.04	99
Kidney wt/100 g body wt	1.22 ± 0.02	0.86 ± 0.03	70‡	0.94 ± 0.04	77‡	1.02 ± 0.02	128‡
Blood glucose (mmol/L)	35.2 ± 3.6	7.10 ± 0.50	20‡	10.0 ± 2.5	28‡	18.5 ± 0.05	370‡
PPP enzyme activities (U/g)							
G6P dehydrogenase	1.76 ± 0.11	0.72 ± 0.06	41‡	0.87 ± 0.07	49‡	1.20 ± 0.04	140‡
6PG dehydrogenase	1.87 ± 0.11	0.90 ± 0.04	48‡	0.89 ± 0.07	48‡	1.23 ± 0.10	134‡
Transketolase	0.81 ± 0.03	0.76 ± 0.03	94	0.88 ± 0.10	109	0.98 ± 0.05	105
Transaldolase	0.99 ± 0.09	0.94 ± 0.05	95	1.08 ± 0.04	109	0.94 ± 0.02	93

Each value is the mean ± SEM of not less than five observations. The percentage changes and Fisher's P-values are given for comparison of alloxan-diabetic rats with nondiabetic alloxan-treated rats loaded with glucose before administration of alloxan and alloxan-diabetic rats treated with insulin; details of treatment are given in MATERIALS AND METHODS. The streptozocin-diabetic group, included for comparison, is shown with percentage changes relative to the control group given in Table 1. Fisher's P-values are shown by: ‡P < 0.001.

TABLE 3
Effect of short-term alloxan diabetes (7 days) on enzymes of the pentose phosphate pathway in rat liver

	Control	Alloxan diabetic (7 days)	AD/C (%)
Body wt (g)	283 ± 11	199 ± 10	70†
Liver wt (g)	13.0 ± 0.5	9.1 ± 0.6	70*
Liver wt/100 g body wt	4.6 ± 0.2	4.6 ± 0.3	100
Blood glucose (mmol/L)	5.1 ± 0.4	28 ± 3	549†
PPP enzyme activities (U/g)			
G6P dehydrogenase	1.43 ± 0.08	1.11 ± 0.07	78*
6PG dehydrogenase	2.71 ± 0.22	1.55 ± 0.20	57*
Transketolase	2.18 ± 0.19	1.81 ± 0.10	83
Transaldolase	0.82 ± 0.01	0.76 ± 0.05	93

The values given are the means ± SEM of six values in each group. Fisher's P-values are shown by: *P < 0.01 and †P < 0.001.

induced diabetes on the activity of two enzymes at the same time interval after the induction of diabetes. In this latter group, there was a raised blood glucose and the activities of G6P dehydrogenase and of 6PG dehydrogenase were also raised above control levels. It may be noted that the effect of STZ in this respect is less marked than that observed with alloxan, as is its effect on the blood glucose level.

The above results directed attention to the correlation between blood glucose, kidney weight, and enzyme activities. Taking the values given in Table 2 for the effect on the various parameters 2 days after the administration of the diabetogenic agent and the values for these same parameters in the control and 2-day diabetic rats in Table 1, positive correlations were shown to exist between blood glucose and kidney wt/100 g body wt ($r = 0.94$, $N = 6$, $P < 0.01$); blood glucose and G6P dehydrogenase ($r = 0.83$, $N = 6$, $P < 0.05$); and blood glucose and 6PG dehydrogenase ($r = 0.86$, $N = 6$, $P < 0.05$). The first of these correlations is in line with the report by Seyer-Hansen.²

Further evidence that the enzyme changes are specifically related to diabetes and are not the resultant of a toxic reaction of alloxan can be drawn from a comparison between the enzyme changes in the kidney with those occurring at the same time in the liver. In this experiment, the comparison was made using animals diabetic for 7 days (Tables 1 and 3). In the kidney, the two dehydrogenases increased more than twofold, and the kidney wt/100 g body wt increased by 67%. In the liver, the enzymes were significantly decreased and the liver wt/100 g body wt remained unchanged.

Metabolite changes in diabetic rat kidney. The tissue content of some substances of special significance to the PPP, either as substrates or as acceptors of the NADPH produced in the pathway (sorbitol and fructose), was measured in the kidneys of rats 7 days after the induction of alloxan diabetes. The results of such measurements are shown in Table 4. The tissue content of all four of the metabolites measured increased. The rise in the content of sorbitol and fructose shows an increased activity of the sorbitol route in line with the known aldose reductase content of this tissue,^{11,12} its high K_m for glucose, and the high intracellular glucose content. The doubling of the G6P content is also significant in relation to the rise in flux through the PPP as shown in Table 2.

DISCUSSION

The oxidative and nonoxidative segments of the PPP provide a flexible and efficient system for the provision of two es-

sential requirements for cellular growth and function, namely, ribose-5-phosphate for nucleotide and nucleic acid synthesis, and NADPH for reductive synthetic reactions and proton formation (see Horecker¹³). Three conditions can be envisaged. First, where there is rapid growth together with a requirement for reducing equivalents, the oxidative reactions of the PPP provides both components. Second, under conditions in which reducing equivalents are the major requirement, the excess pentose phosphate component can be salvaged by reconversion to hexose monophosphate. In the third situation, where NADPH use is minor relative to the rate of nucleic acid synthesis, the requirement for ribose-5-phosphate could be met by the nonoxidative reactions of the route, the oxidative reactions being regulated by the redox state of NADP⁺/NADPH.¹⁴

The kidney, at various time intervals after induction of diabetes, exhibits two distinct patterns of requirement for components of the PPP, and this is reflected in the changing enzyme profiles; these two distinct states are illustrated in Figure 2.

In early diabetes, 0–7 days after alloxan administration, the enzyme profile reflects the increased requirement for ribose-5-phosphate for RNA synthesis associated with the rapid growth processes,^{15–17} and NADPH for reductive synthetic reactions, electrolyte, and acid-base balance changes.^{4,5} There is evidence from this present study that both of these requirements are met by increased flux through the oxidative reactions of the PPP, as shown by the significant rise in G6P dehydrogenase and 6PG dehydrogenase activities, and the fall in transketolase activity. The quotient G6P dehydrogenase/transketolase activity is 0.92 ± 0.05 in normal rat kidney and rises to 2.32 ± 0.21 and 2.47 ± 0.28 at 2 and 7 days, respectively, after induction of diabetes. The fall in transketolase relative to the oxidative enzymes of the pathway could have the effect of decreasing recycling of pentose phosphates to hexosemonophosphate during the phase of rapid growth, increasing the bioavailability of ribose-5-phosphate for phosphoribosylpyrophosphate (PRPP) formation.¹⁵

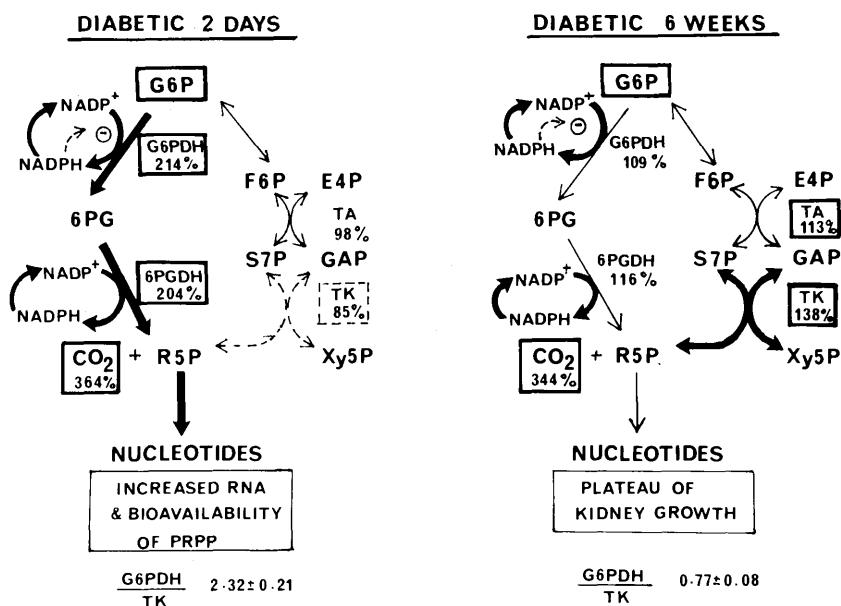
In later stages of diabetes, 21–42 days after alloxan administration, the major growth phase of the kidney is complete,^{1,16,17} while the requirement for NADPH in relation to electrolyte and acid-base balance in the uncontrolled diabetic state remains undiminished. The profile of the PPP at this time interval accords with these changed requirements. Thus, while the flux of glucose through the oxidative reactions

TABLE 4
Effect of alloxan diabetes (7 days) on metabolite content of rat kidney

	Control	Alloxan diabetic (7 days)	AD/C (%)
Body wt (g)	319 ± 10	236 ± 5	74‡
Kidney wt (g)	2.73 ± 0.13	3.66 ± 0.25	134†
Kidney wt/100 g body wt	0.86 ± 0.04	1.55 ± 0.07	180‡
Blood glucose (mmol/L)	4.8 ± 0.4	30 ± 2	625‡
Metabolite content of kidney			
Glucose (μmol/g)	4.4 ± 0.2	28 ± 2	636‡
Glucose-6-phosphate (nmol/g)	49 ± 3	98 ± 6	200‡
Sorbitol (nmol/g)	255 ± 14	320 ± 20	125*
Fructose (nmol/g)	174 ± 9	257 ± 20	148†

The values given are the means ± SEM of 12 values in each group. Fisher's P-values are shown by: *P < 0.05, †P < 0.01, and ‡P < 0.001.

FIGURE 2. Relative activities of the oxidative and nonoxidative segments of the pentose pathway in rat kidney 2 days and 6 wk after the induction of diabetes. □, Enzymes and metabolites increased in diabetes; ▢, enzymes decreased in diabetes; →, enzymes or metabolites increased in diabetes; and ---→, enzymes decreased in diabetes. The percentages shown are the activities of enzymes or yields of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glucose relative to the age-matched control group and calculated from activities/g kidney (see Table 1). The relative activities of the oxidative and nonoxidative segments of the pentose phosphate pathway (PPP) are shown by the quotients of the activities of the rate-limiting enzymes of each segment, glucose-6-phosphate dehydrogenase (G6PDH)/transketolase (TK); these are 0.92 ± 0.05 , 2.32 ± 0.21 , and 0.77 ± 0.08 for normal, 2-day diabetic, and 6-wk diabetic rat kidney cortex, respectively. Abbreviations used are: G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; R5P, ribulose-5-phosphate and ribose-5-phosphate; Xy5P, xylulose-5-phosphate; GAP, glyceraldehyde-3-phosphate; E4P, erythrose-4-phosphate; F6P, fructose-6-phosphate; and TA, transaldolase.



($C_1 - C_6$) remains at 3–4-fold greater than the control value, indicating a higher rate of utilization of NADPH, the enzyme profile reflects the altered fate of ribose-5-phosphate, a greater proportion being reconverted to hexosemonophosphate via transketolase and the G6P dehydrogenase/transketolase quotient now falling to 0.77 ± 0.08 (see Figure 2).

The present biphasic changes in enzyme activities are consistent with published values for changes in RNA and DNA synthesis in rat kidney in experimental diabetes.^{1,16} Further, it is noteworthy that Farquhar et al.¹⁸ described an increased activity of the PPP in kidney hypertrophy after unilateral nephrectomy, with the peak activity of the two dehydrogenases coincident with maximal rates of RNA synthesis.¹⁹ The activity of the dehydrogenases of the PPP was restored to control values when growth processes were essentially complete. In this context, it is interesting to note that NADPH and certain sugar phosphates have been shown to activate protein synthesis in reticulocytes and may exert a similar effect in the hypertrophying kidney.^{20,21}

One of the main questions to emerge is the nature of the factor(s) increasing the rate of the PPP in the diabetic rat kidney. An increased flux in diabetes may be related to the following observed changes. First, there is an increase in the activity of G6P dehydrogenase and 6PG dehydrogenase, although it is unlikely that these changes could, of themselves, account for the fourfold increase of PPP flux (Table 2). Second, the tissue content of G6P increases in diabetes from $49 \pm 3 \mu\text{M}$ to $98 \pm 5 \mu\text{M}$ (Table 4). The apparent K_m for G6P dehydrogenase, purified from adrenal gland, has been reported to be $42 \mu\text{M}$ for G6P.²² Preliminary studies in this laboratory have shown that the apparent K_m for G6P for the kidney enzyme is approximately the same. Thus, in normal kidney, the G6P dehydrogenase is operating around its K_m . Increasing the tissue content of G6P to the level found in the kidney of the diabetic rat must substantially increase the flux of glucose-6-phosphate to pentose phosphate. Third, the PPP requires a supply of NADP⁺ and is subject to feedback inhibition by its product NADPH.¹⁴ The importance of the redox state of the NADPH/NADP⁺ couple in the regulation

of the PPP in kidney is shown by the massive stimulation occurring in this pathway in the presence of the artificial electron acceptor, phenazine methosulphate.⁹ There are a number of systems utilizing NADPH that could be linked to the stimulation of the PPP in the diabetic rat kidney. These include reductive biosyntheses, such as fatty acid synthesis²³ and deoxyribonucleotide synthesis,^{1,2} and the requirement of protons in electrolyte and acid-base balance,⁴ all of which increase in the kidney in diabetes. One system of potential importance examined here is the formation of sorbitol from glucose by aldose reductase, a reaction utilizing NADPH and generating NADP⁺. As shown in Table 4, the products of the sorbitol pathway, sorbitol and fructose, are substantially increased in the diabetic rat kidney in line with the recent work of Beyer-Mears et al.,¹² who have shown a 10-fold increase of sorbitol in the glomeruli from rat kidney after 6 wk of diabetes. Further, the presence of aldose reductase in the kidney has been reported.^{11,12} The properties of this enzyme, with its high K_m for glucose, are such that its activity is greatly enhanced at the high glucose concentration found in the kidney in experimental diabetes.

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