Renal hypertrophy in experimental diabetes

Effect of diabetes on the pathways of glucose metabolism: differential response in adult and immature rats

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

The effect of short-term diabetes, 5 days after the administration of streptozotocin, on renal growth and the activity of alternative pathways of glucose metabolism was studied in immature (21-day-old) rats and in adult rats. The kidney weight increased by 28% in the adult diabetic rats, but by only 10% in the immature diabetic rats, relative to their age-matched control groups. The flux of glucose via the pentose phosphate pathway was increased 2–3-fold in the adult diabetic rats, but was unchanged in the immature diabetic group. Enzymes of this pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) increased by 29% and 77% respectively in adult diabetic rats; in the immature group they showed changes of +5% and +28% respectively. The rate of glucose phosphorylation increased significantly in both groups of diabetic rats; only minor changes were observed in oxidation via the tricarboxylic acid cycle. Increases of 40–50% were found in the activity of enzymes involved in UDP-glucose metabolism (phosphoglucomutase, UDPglucose pyrophosphorylase and UDPglucose dehydrogenase) and in lactate dehydrogenase in both young and adult animals. The results suggest a differential renal response to streptozotocin-diabetes according to the stage of renal growth and development, and it is proposed that the difference is related to the developmental emergence of aldose reductase. Enzymes involved in formation of ribose 5-phosphate and NADPH are strikingly increased in the adult diabetic, whereas metabolic functions dependent on a high ambient glucose concentration, e.g. synthesis of glycogen and glucuronate, are similarly affected in adult and immature diabetic groups, both showing certain aspects of 'glucose overutilization'.

INTRODUCTION

The short-term effects of streptozotocin on renal growth in adult female rats has been studied in detail by Seyer-Hansen (1976, 1983), who showed that, 3 days after the injection of streptozotocin, kidney weight increased by 19%, rising to 39% at 8 days. This author also demonstrated that, during the first 7 days of diabetes, there was a correlation between kidney growth and the blood glucose values over a wide range of blood glucose concentrations (Seyer-Hansen, 1977).

We have previously reported a significant positive correlation between the activity of the pentose phosphate pathway and the extent of renal hypertrophy in adult male rats in both long-term diabetes, which occurred 6 weeks after induction of experimental diabetes with, or without, unilateral nephrectomy (Steer *et al.*, 1982), and in short-term experimental diabetes, in the 2–7 day period after induction of diabetes with alloxan (Steer *et al.*, 1985).

Most investigations into renal hypertrophy to date have concentrated on changes in the adult rat, and less attention has been given to the possible biochemical changes in the immature rat, where growth of the kidney is already occurring at a rapid rate. The immature-rat experimental model may well be of importance in throwing light on the known changes in kidney size and function in early juvenile diabetes (Mogensen, 1971; Mogensen & Andersen, 1973).

The present study examines the effects of streptozotocin-diabetes on kidney growth and the pathways of glucose

utilization in young, newly weaned, rats (aged 21 days) and compares their renal response to diabetes with that of adult rats similarly treated. The enzymes of the pentose phosphate pathway, enzymes involved in the formation and utilization of glucose 6-phosphate and UDP-glucose, and key enzymes of the glycolytic pathway were measured in kidney cortex, together with the flux of ^{14}C - and ^3H -labelled glucose through alternative metabolic routes.

METHODS

Materials

Substrates and coenzymes used in the enzymic assay procedures were purchased either from Boehringer Corp. (Lewes, Sussex, U.K.) or Sigma Chemical Co. (Poole, Dorset, U.K.). Radiochemicals were purchased from The Radiochemical Centre, (Amersham, Bucks., U.K.), except for $[3,4-^{14}\text{C}]$ glucose, which was obtained from New England Nuclear Corp. (Southampton, U.K.).

Animals

Albino male rats of the Wistar strain were used. The newly weaned rats, 21 days old, weighed approx. 50 g at the commencement of the experiment, and the 2-month-old rats approx. 220 g. Streptozotocin, dissolved in citrate buffer, pH 4.0, was administered by intraperitoneal injection, at a dose of 120 mg/kg body wt. Standard pelleted laboratory diet and water were allowed *ad lib.* and, in most experiments, the rats were used 5 days later.

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

Additional groups of 21-day-old rats were studied at 3 days after the streptozotocin treatment. An additional control group, taken at the time of the administration of the diabetogenic agent, i.e. at 21 days, was studied and is termed, in Table 1 and the text, 'newly weaned 21-day-old rats'. The severity of diabetes was evaluated by blood glucose measurement.

Enzyme determinations

Kidney-cortex homogenates (1:5, w/v) were prepared in 0.25 M-sucrose medium buffered with 0.02 M-triethanolamine, pH 7.4, and containing 0.12 mM-dithiothreitol. A high-speed-supernatant fraction was obtained by centrifugation at 105000 g for 45 min; this fraction was dialysed against the same buffer for 1 h at 4 °C. Enzymes of the pentose phosphate pathway, UDP-glucose metabolism and the glycolytic route, as well as blood glucose, were measured as previously described (Novello & McLean, 1968; Gumaa & McLean, 1972; Bergmeyer, 1974; Sochor *et al.*, 1979). Aldose reductase (EC 1.1.1.21) was measured, with glucose as substrate, by the method of Wermuth & von Wartburg (1982) and sorbitol dehydrogenase (EC 1.1.1.14) as described by Bergmeyer (1974).

Flux studies

The conversion of specifically labelled glucose into $^{14}\text{CO}_2$ or $^3\text{H}_2\text{O}$ by kidney slices incubated for 1 h in Krebs-Ringer bicarbonate medium, containing 5 mM- or 20 mM-glucose and 0.5 μCi of labelled substrate, was measured as previously described (Sochor *et al.*, 1979). These were used to provide a medium with glucose concentrations similar to the blood glucose values *in vivo*; 5 mM-glucose was used for immature and adult control kidney slices and 20 mM-glucose for the two groups of diabetic rats. The validity of this procedure has been discussed by Sochor *et al.* (1979). The approximate activity of the pentose phosphate pathway is given by the difference in $^{14}\text{CO}_2$ yields from $[1-^{14}\text{C}]\text{glucose}$ and $[6-^{14}\text{C}]\text{glucose}$ (C1-C6). The flux of glucose through the pyruvate dehydrogenase reaction is indicated by the yield of $^{14}\text{CO}_2$ from $[3,4-^{14}\text{C}]\text{glucose}$, and the oxidation of glucose via the tricarboxylic acid cycle by the $^{14}\text{CO}_2$ yields from $[6-^{14}\text{C}]\text{glucose}$. The phosphorylation of glucose and its conversion into fructose 6-phosphate is evaluated from the $^3\text{H}_2\text{O}$ yields from $[2-^3\text{H}]\text{glucose}$ (Hutton, 1972).

Metabolite determinations

Metabolites were measured in HClO_4 extracts of freeze-clamped kidneys by the standard procedures described by Bergmeyer (1974).

RESULTS

Effect of diabetes on kidney growth

In the adult rat, diabetes resulted in a 27% increase in kidney weight in 5 days. This increase agrees well with the values reported by Seyer-Hansen (1976), where the kidneys of adult fully grown female rats grew by 19% in 3 days and by 39% in 8 days after the induction of diabetes. In contrast, there was only a small increase (10%) in the weight of the kidneys of immature rats 5 days after streptozotocin treatment compared with age-matched controls (Table 1).

Effect of diabetes on the activities of enzymes of the alternative routes of glucose utilization

Previous studies (Steer *et al.*, 1985) have established that the renal growth and elevated enzyme activities found in early streptozotocin-diabetic rats are related to the diabetogenic activity of the drug and not to short-term 'toxic' effects.

Hexokinase. The activity of hexokinase per unit weight of kidney is approximately equal in immature and adult rats. Furthermore, changes in hexokinase activity are essentially similar in immature and adult diabetic rats, with an approx. 14% increase in both groups, statistically significant only in the adult group.

Pentose phosphate pathway. Diabetes (5 days) resulted in an increase in glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in the adult kidney, and in 6-phosphogluconate dehydrogenase (EC 1.1.1.44) and transaldolase (EC 2.2.1.2) in both adult and immature kidneys, whereas transketolase (EC 2.2.1.1), ribose-5'-phosphate isomerase (EC 5.3.1.6) and ribulose-phosphate 3-epimerase (EC 5.1.3.1) remained unchanged in both groups. The net effect of these changes is to approximate the values of the adult kidney towards the profile obtaining in immature, rapidly growing, kidney for 6-phosphogluconate dehydrogenase, transaldolase and transketolase while exaggerating the differences in glucose 6-phosphate dehydrogenase activity (Table 1). It is noteworthy that measurements of enzyme activities in the immature rat kidney at an earlier stage of diabetes (3 days) revealed significant increases in 6-phosphogluconate dehydrogenase (33%; 1.79 ± 0.07 units/g; $P < 0.001$; $n = 6$), transaldolase (13%; 0.91 ± 0.02 unit/g; $P < 0.05$; $n = 6$) and kidney weight (7%; 0.722 ± 0.16 g; $P < 0.05$; $n = 10$), showing that the pattern of response is established early in diabetic hypertrophy.

Glycolytic enzymes. Phosphofructokinase (EC 2.7.1.11), pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27) were measured. Streptozotocin-diabetes (5 days) had no effect on pyruvate kinase activity, but increased phosphofructokinase by 22% in the immature rat kidney and decreased it significantly in the adult. It substantially increased lactate dehydrogenase in both adult and immature kidneys (Table 1). At the earlier stage of 3 days' diabetes, only lactate dehydrogenase activity was raised significantly, and this only in the immature (48%; 78.2 ± 2.5 units/g; $P < 0.001$; $n = 5$).

Enzymes of the UDP-glucose crossroads. All of the three enzymes measured at this locus, phosphoglucomutase (EC 5.4.2.2), UDPglucose pyrophosphorylase (EC 2.7.7.9) and UDPglucose dehydrogenase (EC 1.1.1.22), were substantially increased in the kidneys of diabetic rats, both adult and immature, 5 days after the induction of diabetes. At the shorter time of 3 days' diabetes, only phosphoglucomutase in the immature rat kidney showed a significant increase (40%; 9.87 ± 0.53 units/g; $P < 0.01$; $n = 6$).

Enzymes of the polyol pathway. Neither aldose reductase nor sorbitol dehydrogenase activity was significantly changed as a result of the induction of diabetes (either 5 days or 3 days) in either adult or immature rats.

Table 1. Effect of 5 days' streptozotocin-diabetes on enzymes, metabolites and flux of glucose via alternative metabolic routes in kidney of adult and immature rats

The body weight, kidney weight and blood glucose values are for the entire group of rats used to measure enzymes and flux, 18–24 rats in each group. The numbers of observations for each enzyme or flux measurement are not less than six values. The results are given as means $\pm$ S.E.M.; Fisher's *P* values are given: NS, not significant.

	Newly weaned 21-day-old control group	Immature (26-day-old) rats				Adult (2-month-old) rats			
		Normal (NI)	Diabetic (DI)	100 $\times$ DI/NI	<i>P</i> for NI versus DI	Normal (NA)	Diabetic (DA)	100 $\times$ DA/NA	<i>P</i> for NA versus DA
Initial body wt. (g)	—	48.7 $\pm$ 1.5	49.0 $\pm$ 1.9	101	NS	220 $\pm$ 6	211 $\pm$ 11	96	NS
Final body wt. (g)	47.5 $\pm$ 1.7	60.5 $\pm$ 2.5	49.8 $\pm$ 3.6	82	< 0.05	228 $\pm$ 8	207 $\pm$ 6	91	NS
Final kidney wt. (g)	0.648 $\pm$ 0.035	0.730 $\pm$ 0.018	0.802 $\pm$ 0.018	110	< 0.01	1.98 $\pm$ 0.06	2.51 $\pm$ 0.07	127	< 0.001
Blood glucose (mM)	6.0 $\pm$ 0.4	6.2 $\pm$ 0.4	24.7 $\pm$ 1.7	398	< 0.001	6.1 $\pm$ 0.3	23.5 $\pm$ 1.2	385	< 0.001
Enzyme activity (units/g)									
Pentose phosphate pathway									
Glucose-6-phosphate dehydrogenase	1.09 $\pm$ 0.04	0.99 $\pm$ 0.03	1.04 $\pm$ 0.02	105	NS	1.20 $\pm$ 0.05	1.55 $\pm$ 0.05	129	< 0.001
6-Phosphogluconate dehydrogenase	1.11 $\pm$ 0.04	1.35 $\pm$ 0.09	1.73 $\pm$ 0.09	128	< 0.05	0.87 $\pm$ 0.04	1.54 $\pm$ 0.19	177	< 0.01
Ribose-phosphate isomerase	5.5 $\pm$ 0.4	5.9 $\pm$ 0.4	5.0 $\pm$ 0.3	85	NS	8.5 $\pm$ 1.0	6.2 $\pm$ 0.7	73	NS
Ribulose-phosphate 3-epimerase	17.6 $\pm$ 0.6	15.0 $\pm$ 1.9	16.0 $\pm$ 0.4	107	NS	14.9 $\pm$ 1.7	16.0 $\pm$ 1.2	107	NS
Transketolase	0.86 $\pm$ 0.04	1.03 $\pm$ 0.04	0.97 $\pm$ 0.02	94	NS	0.84 $\pm$ 0.07	1.00 $\pm$ 0.09	119	NS
Transaldolase	0.83 $\pm$ 0.02	0.80 $\pm$ 0.03	1.05 $\pm$ 0.08	131	< 0.05	0.61 $\pm$ 0.05	0.84 $\pm$ 0.03	138	< 0.01
Glycolytic route									
Hexokinase	0.86 $\pm$ 0.08	0.68 $\pm$ 0.04	0.77 $\pm$ 0.04	113	NS	0.59 $\pm$ 0.02	0.67 $\pm$ 0.02	114	< 0.05
Phosphofructokinase	0.73 $\pm$ 0.03	0.97 $\pm$ 0.08	1.19 $\pm$ 0.05	122	NS	2.71 $\pm$ 0.16	2.10 $\pm$ 0.18	77	< 0.05
Pyruvate kinase	14.0 $\pm$ 0.5	14.0 $\pm$ 2.0	13.2 $\pm$ 0.6	94	NS	20.0 $\pm$ 1.0	19.2 $\pm$ 2.2	95	NS
Lactate dehydrogenase	61.9 $\pm$ 2.6	53.0 $\pm$ 1.5	88.5 $\pm$ 10.8	167	< 0.01	63.0 $\pm$ 3.0	100 $\pm$ 4.0	159	< 0.001
UDP-glucose metabolism									
Phosphoglucomutase	7.30 $\pm$ 0.65	7.15 $\pm$ 0.31	10.8 $\pm$ 1.1	151	< 0.01	10.1 $\pm$ 0.7	14.0 $\pm$ 1.0	139	< 0.05
UDPGlucose pyrophosphorylase	2.30 $\pm$ 0.15	2.24 $\pm$ 0.16	3.28 $\pm$ 0.29	143	< 0.05	2.95 $\pm$ 0.13	4.46 $\pm$ 0.51	151	< 0.05
UDPGlucose dehydrogenase	0.132 $\pm$ 0.004	0.160 $\pm$ 0.007	0.217 $\pm$ 0.012	136	< 0.01	0.15 $\pm$ 0.01	0.21 $\pm$ 0.01	139	< 0.05
Polyol pathway									
Aldose reductase	0.054 $\pm$ 0.004	0.076 $\pm$ 0.007	0.080 $\pm$ 0.008	105	NS	0.149 $\pm$ 0.011	0.133 $\pm$ 0.009	89	NS
Sorbitol dehydrogenase	5.22 $\pm$ 0.17	4.74 $\pm$ 0.36	4.93 $\pm$ 0.38	104	NS	5.47 $\pm$ 0.24	5.37 $\pm$ 0.17	98	NS
Flux of glucose (μ mol/h per g)									
[1- 14 C]Glucose $\rightarrow$ 14 CO ₂	4.56 $\pm$ 0.45	4.67 $\pm$ 0.34	4.92 $\pm$ 0.15	105	NS	4.51 $\pm$ 0.26	4.83 $\pm$ 0.37	107	NS
[3,4- 14 C]Glucose $\rightarrow$ 14 CO ₂	6.70 $\pm$ 0.59	8.50 $\pm$ 0.18	8.54 $\pm$ 0.48	100	NS	6.35 $\pm$ 0.40	6.14 $\pm$ 0.64	97	NS
[6- 14 C]Glucose $\rightarrow$ 14 CO ₂	3.89 $\pm$ 0.52	4.11 $\pm$ 0.20	4.42 $\pm$ 0.19	107	NS	3.91 $\pm$ 0.18	3.56 $\pm$ 0.09	91	NS
[2- 3 H]Glucose $\rightarrow$ 3 H ₂ O	8.04 $\pm$ 0.34	9.24 $\pm$ 0.56	12.0 $\pm$ 0.47	130	< 0.01	8.51 $\pm$ 0.43	11.7 $\pm$ 0.76	140	< 0.01
Approx. pentose phosphate pathway flux (C1–C6)	0.67 $\pm$ 0.17	0.56 $\pm$ 0.15	0.50 $\pm$ 0.12	89	NS	0.60 $\pm$ 0.15	1.27 $\pm$ 0.18	212	< 0.05

Effect of diabetes on the flux of glucose through alternative metabolic pathways

The conversion of glucose labelled on C-1, C-6 or C-3,4 into $^{14}\text{CO}_2$ and of $[2\text{-}^3\text{H}]\text{glucose}$ into $^3\text{H}_2\text{O}$ by kidney slices from adult and immature normal and diabetic rats, the latter 5 days after streptozotocin treatment, was measured to obtain an approximate assessment of the relative changes in metabolic routes, including: glucose phosphorylation ($^3\text{H}_2\text{O}$ from $[2\text{-}^3\text{H}]\text{glucose}$); the pentose phosphate pathway ($^{14}\text{CO}_2$ yield from $[1\text{-}^{14}\text{C}]\text{glucose}$ — $^{14}\text{CO}_2$ yield from $[6\text{-}^{14}\text{C}]\text{glucose}$); the pyruvate dehydrogenase reaction ($^{14}\text{CO}_2$ yield from $[3,4\text{-}^{14}\text{C}]\text{glucose}$) and the tricarboxylic acid cycle ($^{14}\text{CO}_2$ yield from $[6\text{-}^{14}\text{C}]\text{glucose}$). The major difference between the normal immature rat and the adult rat kidney was the faster rate of conversion of $[3,4\text{-}^{14}\text{C}]\text{glucose}$ into $^{14}\text{CO}_2$ by the immature rat kidney, indicative of a faster rate of the pyruvate dehydrogenase reaction (Table 1). Since the phosphorylation of glucose, oxidation in the pentose phosphate pathway and in the tricarboxylic acid cycle were all essentially similar in these two groups, the difference in $^{14}\text{CO}_2$ yield from $[3,4\text{-}^{14}\text{C}]\text{glucose}$ may be interpreted as showing a faster rate of acetyl-group formation from glucose and its diversion to biosynthetic purposes in the immature rat kidney. A similar difference was observed in the two diabetic groups.

The adult and immature rat kidney showed a common pattern of increase in the rate of formation of $^3\text{H}_2\text{O}$ from $[2\text{-}^3\text{H}]\text{glucose}$ in diabetes. The increase of 30–40% was similar in the two groups (Table 1). A marked point of contrast in the response of the adult and immature rat kidney to diabetes was seen in the activity of the pentose phosphate pathway (C1–C6), which increased 2-fold in the adult diabetic rat kidney but remained unchanged in the immature diabetic rat kidney relative to the age-matched control groups.

DISCUSSION

Diabetes-induced kidney growth in adult and immature rats

The increase in kidney weight in the early stages of diabetes differs in adult and immature rats in that, in the former case, the entire growth can be ascribed to diabetes-induced renal hypertrophy, whereas in the latter case the renal hypertrophy is superimposed on normal developmental growth. When allowance is made for development, it can be calculated that renal hypertrophy in the adult adds 0.054 g/day per g of kidney, whereas in the immature rat the corresponding value is 0.019 g/day per g of kidney, i.e. the response of the adult kidney to diabetes is almost 3 times that of the immature.

Glucose utilization in relation to kidney growth in diabetes

The renal changes in adult and immature rats after the induction of diabetes can be separated into two broad categories; the first occur as a common response to diabetes in the two age groups, whereas the second relate to specific increases in key enzymes and pathways in one, but not both, groups. It is not unreasonable to propose, by analogy with the constant and specific proportion studies by Pette *et al.* (1962) and Pette (1966), that these latter changes are more related to the differential growth pattern.

(a) **Common responses to diabetes in adult and immature rats.** This group includes enzymes such as phosphoglucumutase, UDPglucose pyrophosphorylase and UDPglucose dehydrogenase, which are involved in complex-carbohydrate synthesis, e.g. glycogen and components of the basement membrane (Needleman *et al.*, 1968; Spiro, 1976; Cortes *et al.*, 1982; Spiro, 1984).

Also common to the two groups is the increase in glucose phosphorylation, indicative of some degree of 'glucose overutilization', although it should be observed that the flux through glycolysis and the tricarboxylic acid cycle is not increased by diabetes in either age group.

(b) The differing response of the pentose phosphate pathway to diabetes in the kidney of the adult and immature rat.

Early renal hypertrophy in the adult diabetic rat has been shown to be positively correlated with: (i) the blood glucose concentration (Seyer-Hansen, 1977); (ii) the flux of glucose through the oxidative segment of the pentose phosphate pathway (Steer *et al.*, 1982, 1985); and (iii) the activity of glucose-6-phosphate dehydrogenase (Steer *et al.*, 1985). The present observations that there is (i) a differing, and slower, rate of diabetes-induced growth in the immature rat relative to the adult rat kidney, despite closely similar degrees of hyperglycaemia, (ii) no increase in the flux of glucose through the oxidative segment of the pentose phosphate pathway in the immature rat kidney in diabetes, in contrast with the adult, and (iii) no increase in the activity of glucose-6-phosphate dehydrogenase or in glucose 6-phosphate concentration in the immature rat kidney in diabetes [control, 19.7 ± 4.1 ($n = 9$); diabetic, 26.8 ± 4.2 ($n = 12$) (nmol/g)], again in contrast with the adult, strongly suggest that the pentose phosphate pathway is intimately linked to renal growth processes in diabetes and that understanding of the regulation of this pathway in kidney could clarify the processes leading to renal hypertrophy.

The differing pattern of response in the kidney of the adult normal and diabetic animals is seen mainly in the enzymes of the oxidative, rather than the non-oxidative, segment of the pentose phosphate pathway (Table 1) which indicates that the link between this pathway and growth resides in this segment of the pathway. Probably the most potent factor regulating the activity of this segment is the concentration of NADPH, a powerful inhibitor of glucose-6-phosphate dehydrogenase (Krebs & Eggleston, 1974).

All three parameters raised in the adult kidney in diabetes, i.e. activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and glucose 6-phosphate content [control, 48 ± 3 ($n = 8$), diabetic, 97 ± 6 ($n = 8$) nmol/g; $P < 0.001$] could contribute to the increased flux through the pentose phosphate pathway. Equally, the failure of the flux through the pathway to increase in the immature rat kidney in short-term diabetes could be ascribed to the unchanged glucose-6-phosphate dehydrogenase activity and low concentration of glucose 6-phosphate. Nevertheless, in view of the fact that, in the absence of a system for the reoxidation of NADPH, only a small fraction (approx. 5%) of the potential activity of the pentose phosphate pathway is expressed, changes in glucose-6-phosphate dehydrogenase and glucose 6-phosphate alone are unlikely substantially to change the flux in the

pathway unless accompanied by a parallel increase in a NADPH-acceptor system (Sochor *et al.*, 1979).

The major NADPH-utilizing system in adult rat kidney which is dependent on high blood glucose concentrations, such as those found in experimental diabetes, is aldose reductase, the K_m of which is reported to be 51 mM for kidney (Cromlish & Flynn, 1983). Although this enzyme would make a very small contribution to NADPH reoxidation in either adult or immature rat kidney at normal blood glucose concentrations, it could have a substantial effect at blood glucose values of approx. 25 mM. In this context it is significant that aldose reductase activity (with glucose as substrate) shows a developmental pattern, being lower in immature rats than in adults. Thus the extent to which aldose reductase can contribute NADP⁺, required for the activity of the pentose phosphate pathway, is age-dependent and can be calculated to account for the oxidation of approx. 3 μ mol of NADPH/h per g at 37°C in the adult and 1.2–1.9 μ mol/h per g in the immature kidney. These values compare well with the flux observed (Table 1) for adult diabetic rats, which is equivalent to 2.54 μ mol of NADPH produced/h per g at 37°C, and 1.0 μ mol of NADPH/h per g at 37°C for immature rats.

The linking of the pentose phosphate pathway to high blood glucose values via aldose reductase may be a common feature of glucose overutilization in a number of tissues not dependent on insulin for glucose uptake, as originally shown in lens by Kinoshita *et al.* (1963). Similarly, n.m.r. studies show increased sorbitol formation and turnover of NADPH in lens and a 5-fold increase in the flux of glucose through the PPP with elevated glucose concentrations (Gonzalez *et al.*, 1984). It should thus appear that the mechanism of the different growth responses to diabetes in adult and immature rats stems, in part, from the developmental pattern of aldose reductase.

There still remains the question of the relationship between increased pentose-phosphate-pathway activity and renal growth in diabetes, a link required in understanding the positive correlation between increased blood glucose, NADPH reoxidation by aldose reductase, increased pathway activity and kidney growth. This may be related to an increased formation of ribose 5-phosphate by the oxidative pentose phosphate pathway and the production of phosphoribosyl pyrophosphate. The importance of this locus has been shown by the studies of Cortes *et al.* (1980), who have demonstrated increased bioavailability of phosphoribosyl pyrophosphate in early renal growth in diabetes. The relationship between formation and utilization of phosphoribosyl pyrophos-

phate in diabetes at different development stages is the subject of the following paper (Kunjara *et al.*, 1986).

We thank the British Diabetic Association, the Basil Samuel Charitable Trust and the National Kidney Research Fund for their generous support. S.K. was supported by the British Council.

REFERENCES

- Bergmeyer, H.-U. (1974) *Methods in Enzymatic Analysis*, 2nd edn., Academic Press, New York
- Cortes, P., Verghese, C. P., Venkatachalam, K. K., Schoenberger, A. M. & Levin, N. W. (1980) *Am. J. Physiol.* **238**, 341–348
- Cortes, P., Dumler, F., Sury-Sastry, K. S., Verghese, C. P. & Levin, N. W. (1982) *Kidney Int.* **21**, 676–682
- Cromlish, J. A. & Flynn, T. G. (1983) *J. Biol. Chem.* **258**, 3583–3586
- Gonzalez, R. G., Barnett, P., Aguayo, J., Cheng, H.-M. & Chylack, L. T., Jr. (1984) *Diabetes* **33**, 196–199
- Gumaa, K. A. & McLean, P. (1972) *FEBS Lett.* **27**, 293–297
- Hutton, J. J. (1972) *Anal. Biochem.* **45**, 577–584
- Kinoshita, J. H., Futterman, S., Satoh, K. & Merola, L. O. (1963) *Biochim. Biophys. Acta* **74**, 340–350
- Krebs, H. A. & Eggleston, L. V. (1974) *Adv. Enzyme Regul.* **12**, 421–434
- Kunjara, S., Sochor, M., Adeoya, A., McLean, P. & Greenbaum, A. L. (1986) *Biochem. J.* **234**, 579–585
- Mogensen, C. E. (1971) *Scand. J. Clin. Lab. Invest.* **28**, 79–90
- Mogensen, C. E. & Andersen, M. J. F. (1973) *Diabetes* **22**, 707–712
- Needleman, P., Passonneau, J. V. & Lowry O. H. (1968) *Am. J. Physiol.* **215**, 655–659
- Novello, F. & McLean, P. (1968) *Biochem. J.* **107**, 775–791
- Pette, D. (1966) in *Regulation of Metabolic Processes in Mitochondria* (Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C., eds.), pp. 28–50, Elsevier, Amsterdam
- Pette, D., Luh, W. & Bucher, Th. (1962) *Biochem. Biophys. Res. Commun.* **7**, 419–424
- 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
- Spiro, M. J. (1984) *Diabetologia* **26**, 70–75
- Spiro, R. G. (1976) *Diabetologia* **12**, 1–14
- 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
- Wermuth, B. & von Wartburg, J. P. (1982) *Methods Enzymol.* **89**, 181–186