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Gluconeogenesis in Developing Rat Kidney Cortex

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1. Gluconeogenesis in developing rat kidney cortex was studied by assaying the activities of two enzymes, glucose 6-phosphatase and phosphoenolpyruvate carboxykinase, and by measuring glucose formation in tissue slices. 2. Glucose 6-phosphatase and phosphoenolpyruvate carboxykinase are present in late foetal (21–22-day-old) tissue and increase rapidly postnatally. Maximum activity of phosphoenolpyruvate carboxykinase occurs at 7 days of age, followed by a decline to the adult level. Glucose 6-phosphatase activity rises during the first 2 postnatal weeks and then declines. 3. Late foetuses synthesize glucose from both pyruvate and L-glutamate. The rate increases during the first 2 weeks to above adult levels. Synthesis is always higher from pyruvate than from glutamate. 4. The effect of 24 hr. starvation was studied in perinatal animals. The results indicate that the ability to increase the rate of glucose synthesis as a result of starvation is not present at birth, but develops some time after the second postnatal day.

The overall gluconeogenic capacity of mammalian liver has been investigated as a function of developmental age (Ballard & Oliver, 1963, 1965; Yarnell, Nelson & Wagle, 1966; Yeung & Oliver, 1967*a*,*b*; Vernon, Eaton & Walker, 1967). In addition there is information on the prenatal and postnatal development of enzymes of liver gluconeogenesis in a variety of species (see review by Dawkins, 1966; Zorzoli, 1962; Burch *et al.* 1963; Nelson, Yarnell & Wagle, 1966; Ballard & Hanson, 1967; Greengard & Dewey, 1967; Wallace & Newsholme, 1967; Yeung, Stanley & Oliver, 1967).

It is well known that, at least *in vitro*, kidney-cortex tissue also has a high capacity for gluconeogenesis (Krebs, 1964). The development of renal gluconeogenesis has, however, received little attention (Kretchmer, 1959; Dawkins, 1961; Zorzoli, 1968). The present paper reports the results of a study of gluconeogenesis in rat kidney in the late foetal stage, at birth and during the first month of life. Glucose production by tissue slices was studied in relation to the activities of two enzymes of gluconeogenesis, glucose 6-phosphatase (D-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9) and phosphoenolpyruvate carboxykinase [GTP–oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32]. In addition the effect of 24 hr. starvation on glucose synthesis in perinatal animals was examined.

MATERIALS AND METHODS

Animals. Wistar-strain rats born and raised in this Laboratory were used. Foetuses of known age were obtained by mating the females overnight and assuming that conception occurred at the midpoint of the mating period. At the time of being killed the pregnant females were placed under light ether anaesthesia, and foetuses were removed and immediately weighed, measured and killed. Foetal body weights ranged from 2·0 to 5·7g. and crown–rump lengths varied from 32 to 41mm. Litters were kept with their mothers but were reduced in size so that each lactating female nursed only eight pups. The young were weaned at 21 days.

Measurement of glucose production. All animals were weighed, decapitated and exsanguinated. Both kidneys were removed and immediately weighed and chilled. Foetal and up to 2-day-old kidneys were sliced under a dissecting microscope with micro-surgery instruments. Older kidneys were sliced with a Stadie–Riggs microtome. The procedures for handling of slices, incubation and assay of glucose have been reported (Zorzoli & Li, 1967; Zorzoli, 1968). They were adapted from the methods used by Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963) and Rutman, Meltzer, Kitchell, Rutman & George (1965). Glucose content was determined quantitatively by using Glucostat, a commercial product (Worthington Biochemical Corp., Freehold, N.J., U.S.A.) containing freeze-dried preparations of glucose oxidase and peroxidase together with a chromogen. Deproteinized portions of the incubation medium were allowed to react with Glucostat reagent for 30min. at 37°. The extinction was measured at 530mμ with a Beckman DU spectrophotometer. Standard glucose solutions and a reagent blank were used in each experiment. To increase the sensitivity of the method, 1ml. of 58% (w/v) H_2SO_4 was added immediately after the 30min. incubation period (Rutman *et al.* 1965).

Enzyme assays. Kidney-cortex slices were blotted free of moisture, weighed and homogenized in 0·25M-sucrose (20mg. wet wt. of tissue/ml. of homogenate). Portions of the whole homogenates were frozen in separate vials and were stored at −80°.

Glucose 6-phosphatase was assayed as reported by

Zorzoli (1962) except that incubation was at 37° for 20min. The P_i liberated from glucose 6-phosphate was determined by the method of Fiske & Subbarow (1925). Tissue blank values were low and did not limit the accuracy of the method. Protein was determined as described by Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard. Activity was calculated as μmoles of P_i formed/g. of protein/min. at 37°.

Portions of whole homogenates were centrifuged in a Spinco model L preparative ultracentrifuge at 105000g for 1hr. at 4°. The supernatant fraction was removed and stored at −80°. PEP* carboxykinase activity was determined in the supernatant by the method of Nordlie & Lardy (1963). A 0·5ml. portion of supernatant was incubated for 7min. at 30° in a final reaction volume of 1·5ml. containing 1·6μmoles of GSH, 9·0μmoles of ITP (Sigma Chemical Co., St Louis, Mo., U.S.A.), 6·7μmoles of oxaloacetic acid, 22·5μmoles of $MgCl_2$, 20μmoles of NaF and 44μmoles of tris–HCl buffer (Sigma Chemical Co.), pH8·0. Each assay was performed in duplicate. The reaction was stopped by the addition of 1ml. of 10% (w/v) trichloroacetic acid; after centrifugation the reaction mixture was assayed for the PEP formed in the presence of the enzyme. Two 0·5ml. samples of each reaction mixture were transferred to large Pyrex test tubes. One sample was treated with 1ml. of 0·25M-$HgCl_2$ (as recommended by Ray, Foster & Lardy, 1964), which selectively hydrolyses PEP. The other sample received 1ml. of water and served as an unhydrolysed blank. Duplicate water blanks and duplicate standards, containing 0·4μmole of P_i, were used with both the hydrolysed and unhydrolysed preparations.

After 18min. of hydrolysis, 6·5ml. of water was added to each tube and the P_i was assayed by the Sumner (1944) method as follows: 1ml. of 2·5M-ammonium molybdate in 5N-H_2SO_4 was added to each tube, followed by 1ml. of 10% (w/v) $FeSO_4,7H_2O$ in 0·1N-H_2SO_4, made up freshly just before use. After 15min. the extinction was measured at 660mμ. Very accurate timing was essential, because ITP is hydrolysed in acid and the extinction changes with time. The concentration of P_i in each tube was calculated, and the difference between the values for the hydrolysed and unhydrolysed portions of each reaction mixture was used to determine the amount of PEP formed in the assay. Activity of the enzyme was expressed as μmoles of PEP formed/g. of supernatant protein/min.

Owing to the presence of ITP in the reaction mixture the unhydrolysed blanks contained a large amount of P_i and the difference between the values for the hydrolysed and unhydrolysed samples was small. In our assays the amount of P_i liberated from PEP ranged from about 45% of the blank values in tissue from adult animals to 7% in tissue from newborn animals, whereas with foetal tissue the difference was extremely small, about 3%. It was our practice to assay tissue from animals of different ages at the same time. The blank values did not vary from age to age and were very uniform within each assay. Therefore, despite the small amount of P_i liberated in foetal tissue, it was considered that enzyme activity was being detected even in this case.

The subcellular distribution of PEP carboxykinase activity in liver, at least, varies with the species studied (Nordlie & Lardy, 1963). It is also influenced by developmental age (Ballard & Hanson, 1967). In our study of rat kidney cortex we did not attempt to assay PEP carboxykinase in the particulate fractions. Preliminary work showed that activity was higher in soluble fractions than in whole homogenates and that the ratio of soluble-fraction activity to homogenate activity did not seem to change with postnatal age. However, the particulate fraction may contain some PEP carboxykinase, and it would be desirable to investigate possible developmental changes in this fraction.

* Abbreviation: PEP, phosphoenolpyruvate.

RESULTS

Glucose production was measured in rat kidney-cortex slices in the presence of two different

Table 1. *Glucose production in foetal and postnatal rat kidney-cortex slices*

Kidney-cortex slices representing 2–6mg. dry wt. were incubated for 1hr. at 40°. The substrate concentration was 10mM. The results are given as means ± S.E.M., with the numbers of determinations in parentheses. Each determination on foetal and 1-, 2-, 3- and 4-day-old animals was made on a pool of tissue taken from two to four animals. At 7 days of age and older single animals were used. Glucose production without added substrate by tissue from animals of different postnatal ages was 17·4 ± 2·3 μmoles of glucose formed/g. dry wt./hr.

	Age (days)	Substrate ...	Glucose formed (μmoles/g. dry wt./hr.) Pyruvate	L-Glutamate
Foetal	21–22		50·1 ± 4·2 (6)	21·8 ± 1·5
Postnatal	1		110·1 ± 5·8 (5)	47·7 ± 2·1
	2		130·4 ± 4·5 (4)	54·8 ± 4·5
	3		144·3 ± 2·1 (4)	50·8 ± 4·0
	4		132·1 ± 2·4 (3)	53·8 ± 1·9
	7		166·5 ± 7·5 (6)	62·0 ± 2·9
	8		191·0 ± 9·7 (4)	62·2 ± 1·9
	16		230·5 ± 8·1 (6)	104·7 ± 6·8
	22		225·0 ± 5·7 (9)	92·1 ± 5·3
	30		205·1 ± 11·4 (4)	97·2 ± 8·0
Adult			154·3 ± 5·8 (12)	94·1 ± 4·3

Table 2. *Activities of glucose 6-phosphatase and PEP carboxykinase in foetal and postnatal rat kidney cortex*

Activities are given as means ± S.E.M., with the numbers of determinations in parentheses. Animals from different litters were used at each age. Each assay on foetal, newborn and 2-day-old animals was made on a pool of tissue taken from two to five animals. At 4 days of age and older single animals were used. Glucose 6-phosphatase and PEP carboxykinase activities were measured in whole homogenates and in 105000*g* supernatants respectively. —, Not determined.

		Activity (μmoles of product formed/g. of protein/min.)	
	Age (days)	Glucose 6-phosphatase	Phosphoenolpyruvate carboxykinase
Foetal	21–22	8·6 ± 1·2 (10)	14 ± 3 (10)
Newborn	(10–180 min.)	19·9 ± 2·7 (12)	51 ± 5 (11)
Postnatal	2	60·0 ± 4·3 (9)	105 ± 16 (8)
	4	79·2 ± 3·2 (6)	—
	7	94·2 ± 3·4 (6)	149 ± 21 (9)
	14	—	95 ± 5 (4)
	16	122·6 ± 6·5 (5)	—
	18	—	94 ± 10 (4)
	22	121·3 ± 8·0 (6)	88 ± 9 (4)
	30	107·0 ± 5·3 (3)	95 ± 11 (3)
Adult		83·3 ± 5·0 (12)	101 ± 6 (16)

Table 3. *Effect of 24 hr. starvation on glucose production by kidney-cortex slices from perinatal animals*

Glucose production in fed and starved animals was calculated as μmoles of glucose formed/g. dry wt. of kidney cortex/hr. incubation at 40°. Each starved animal was compared with its own fed control and the value was expressed as the percentage of the control value. Results are given as means ± S.E.M. and the numbers of pairs of fed and starved animals are indicated in parentheses.

Age when killed (days)	Substrate ...	100 × Starved/fed glucose production ratio	
		Pyruvate	L-Glutamate
1		105 ± 8 (5)	93 ± 10
2		101 ± 11 (4)	104 ± 11
3		143 ± 6 (5)	150 ± 11
4		147 ± 10 (3)	132 ± 9
5		124 ± 5 (5)	123 ± 6
8		128 ± 4 (4)	140 ± 3

substrates, pyruvate and L-glutamate. The animals ranged in age from late foetal (21–22 days) to the thirtieth postnatal day. The results in Table 1 show that late foetuses have the ability to synthesize glucose, although at a lower rate than in the adult (Fig. 1). Developmental increase in glucose synthesis takes place during the first 2 weeks of life, but the sharpest changes occur immediately after birth.

The results for glucose 6-phosphatase at different developmental ages are given in Table 2. Significant activity occurs in foetal kidney cortex. At birth, 10 min. to 3 hr. after natural delivery, activity is more than double that in the foetuses. It continues to rise, reaching maximum activity by the beginning of the third week of life.

Table 2 shows the development of PEP carboxykinase. This enzyme also is present in foetal tissue, but is higher in activity immediately after birth. Activity reaches adult values by 2 days of age and continues to rise to a maximum at 7 days of age (Fig. 1). During the second week activity declines to the adult level.

Table 3 shows the effect of 24 hr. starvation on glucose production by kidney-cortex slices from perinatal animals. Litter mates were arranged in pairs according to body weight ±0·5 g. One member of each pair was left with the mother; the other was separated from the mother for 24 hr. and was maintained at an ambient temperature of 30°. This is approximately the temperature in the nest when the mother is present. It was used because infant rats do not have fully developed thermoregulatory mechanisms, and decline in body temperature is believed to contribute to mortality (Braun & Mosinger, 1958) and to change in blood glucose concentration during starvation (Hahn & Koldovský, 1966).

It is known that starvation of adult animals for various periods of time results in an increase in the rate of renal gluconeogenesis (Krebs *et al.* 1963; Henning, Stumpf, Ohly & Seubert, 1966; Zorzoli & Li, 1967; Underwood & Newsholme, 1967). The present experiment demonstrates that infant rats

starved for the 24 hr. period immediately after birth or between the first and second day of life do not show an increase in kidney gluconeogenesis (Table 3). Animals starved between the second and third day or at older ages show a significant increase in glucose synthesis. Thus it appears that the ability to increase the rate of glucose synthesis as a result of starvation is not present in the rat at birth, but develops some time after the second postnatal day.

DISCUSSION

It was pointed out by Krebs (1964) that kidney-cortex slices suspended in saline constitute a particularly suitable system *in vitro* for studying the rates of gluconeogenesis under different physiological conditions. The system described by Krebs *et al.* (1963) was used in the present work to investigate the development of kidney gluconeogenesis in the rat. It was found that the kidney of the late foetus can synthesize glucose from either pyruvate or L-glutamate, although at a low rate, and that developmental increases to above adult levels occur during the first 2 postnatal weeks. The developmental patterns of gluconeogenesis from the two substrates are generally similar (Fig. 1), although glucose production from glutamate does not rise as high above adult levels as that from pyruvate. Also, the rate of glucose synthesis is always higher from pyruvate than from glutamate (Table 1).

Gluconeogenesis occurs both in liver and in kidney cortex, but the evidence indicates that there is a temporal difference in the development of gluconeogenic capacity in the two tissues. According to Ballard & Oliver (1963, 1965) and Yeung *et al.* (1967) the foetal rat liver does not synthesize glucose or glycogen. Foetal rat kidney cortex, on the other hand, synthesizes glucose at a significant rate (Table 1).

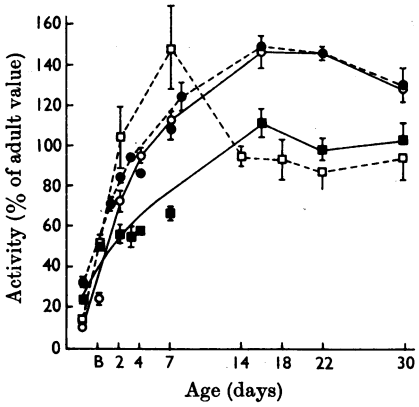

Fig. 1. Development of glucose 6-phosphatase (○), PEP carboxykinase (□) and glucose production from pyruvate (●) and from L-glutamate (■) in rat kidney cortex. Each point is a mean value calculated as a percentage of the adult value. The vertical bars represent the S.E.M. values where they are large enough to be shown. B, Birth.

Only two enzymes were studied here. However, since glucose synthesis occurs in foetal kidney cortex all the necessary enzymes must be present. In liver, glucose 6-phosphatase, fructose 1,6-diphosphatase (D-fructose 1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) and pyruvate carboxylase [pyruvate–carbon dioxide ligase (ADP), EC 6.4.1.1] occur prenatally (Yeung *et al.* 1967); PEP carboxykinase, like the overall process glucose synthesis, appears only after birth (Yeung *et al.* 1967; Ballard & Oliver, 1963, 1965). It may well be that the difference in overall gluconeogenic capacity between foetal liver and foetal kidney stems from the difference in time of initiation of PEP carboxykinase synthesis in the two tissues.

Although liver and kidney cortex possess what appear to be similar biochemical mechanisms for gluconeogenesis, *a priori* there are no reasons for believing that these mechanisms are controlled in identical fashion. Indeed, there is some evidence that in the adult they are not (Weber, 1963). There is no information about the nature of the factor controlling the initiation of PEP carboxykinase synthesis in kidney, but it is clear that it is not associated with the events surrounding normal birth since the enzyme is already present *in utero*. The liver enzyme, on the other hand, is affected by the events of birth and can be induced by prematurely delivering foetuses and maintaining them in an incubator (Yeung & Oliver, 1967*b*).

The rat is probably not unique with respect to the occurrence of prenatal capacity for kidney gluconeogenesis. It has been shown that in the guinea pig both PEP carboxykinase and glucose synthesis from oxaloacetate or succinate are at above adult levels a few hours after birth (Zorzoli, 1968). Since the guinea pig has a long gestation period and is self-sufficient at birth, it is likely that the development of gluconeogenesis starts *in utero*. The mouse, which is more like the rat in gestation time and state of maturity at birth, also synthesizes glucose a few hours after birth and also very probably prenatally (Zorzoli, 1968).

Fig. 1 shows that glucose synthesis from pyruvate and L-glutamate by rat kidney increases rapidly in the first 2 days of life. During this period, however, the infant rat cannot elevate the rate of gluconeogenesis (as a result of starvation) above the level found in the fed controls (Table 3). Previous work from this Laboratory demonstrated that in adult rats renal glucose production and PEP carboxy-

kinase activity are increased after starvation, whereas glucose 6-phosphatase and fructose 1,6-diphosphatase activities decline (Zorzoli & Li, 1967). Henning *et al.* (1966) reported increases in kidney PEP carboxykinase activity and glucose synthesis after both 72hr. starvation and cortisol treatment. These observations suggest a relationship between the activity of kidney PEP carboxykinase and the rate of the overall process. Our present findings show that at birth PEP carboxykinase activity is low, but rises rapidly and reaches the adult value by the second postnatal day. It is only after this age that the starvation-induced elevation of glucose production is observed; the implication is that PEP carboxykinase activity must be at the adult level before starvation can cause an increase in glucose production.

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