Biochem. J. (1972) **127**, 521–529
Printed in Great Britain

Glucose Metabolism in the Developing Rat

STUDIES *IN VIVO*

By RICHARD G. VERNON* and DERYCK G. WALKER
Department of Biochemistry, University of Birmingham, P.O. Box 363, *Birmingham B*15 2*TT, U.K.*

(*Received* 25 *October* 1971)

1. The specific radioactivity of plasma D-glucose and the incorporation of ^{14}C into plasma L-lactate, liver glycogen and skeletal-muscle glycogen was measured as a function of time after the intraperitoneal injection of D-[6-^{14}C]glucose and D-[6-^{3}H]glucose into newborn, 2-, 10- and 30-day-old rats. 2. The log of the specific radioactivity of both plasma D-[6-^{14}C]- and D-[6-^{3}H]-glucose of the 2-, 10- and 30-day-old rats decreased linearly with time for at least 60min after injection of labelled glucose. The specific radioactivity of both plasma D-[6-^{14}C]- and D-[6-^{3}H]-glucose of the newborn rat remained constant for at least 75min after injection. 3. The glucose turnover rate of the 30-day-old rat was significantly greater than (approximately twice) that of the 2- and 10-day-old rats. The relative size of both the glucose pool and the glucose space decreased with age. Less than 10% of the glucose utilized in the 2-, 10- and 30-day-old rats was recycled via the Cori cycle. 4. The results are discussed in relationship to the availability of dietary glucose and other factors that may influence glucose metabolism in the developing rat.

The suckling rat consumes a high-fat, low-carbohydrate diet (Dymsza *et al.*, 1964), whereas the diet given to the adult rat normally contains 60–70% carbohydrate. Because the concentration of ketone bodies and free fatty acids (Drahota *et al.*, 1964; Hahn *et al.*, 1963; Page *et al.*, 1971) in the blood is elevated during the suckling period while certain relevant enzymic activities are low, the rate of carbohydrate utilization may be comparatively low in the suckling animal (Hahn & Koldovský, 1966, p. 76; Vernon & Walker, 1968). Determinations of the rate of glucose utilization in the suckling rat have not been made previously, however, to test this hypothesis.

The capacities of the liver and kidney to synthesize glucose from amino acids, lactate, pyruvate and other organic acids have been shown to increase rapidly after birth in the rat, and to attain maximum values during the suckling period (Ballard & Oliver, 1963; Yeung & Oliver, 1967; Vernon *et al.*, 1968; Zorzoli *et al.*, 1969). Because of the large requirements for protein synthesis, it has been suggested that amino acids may not be readily available for gluconeogenesis in the neonatal rat (Hahn *et al.*, 1961; see also Miller, 1969). Hence, the increased capacity of the pathway from pyruvate to glucose in the suckling rat might reflect an increase in the rate of glucose recycling via the Cori cycle, as has been shown to be the situation in starved rats (Von Holt *et al.*, 1961; Dunn *et al.*, 1967). The work presented in the present paper was designed to measure the glucose turnover rate and the proportion of glucose recycled via the Cori cycle in newborn and in young rats aged 2, 10 and 30 days.

* Present address: Banting and Best Dept. of Medical Research, University of Toronto, Toronto 101, Canada.

Materials and Methods

Animals

The rats were an albino Wistar strain; the normal dietary and weaning regimens were as described by Vernon & Walker (1968). After removal from the mother, 2-day-old and 10-day-old rats were kept in a humid incubator at 30°C until they were killed. The newborn rats used in this study were born naturally; 12 pups were removed from the mother as soon as the last had been born and kept as for the other animals. The birth of the litter usually lasted about 1h; zero time in the experiments on these animals was approx. 15min after removal from the mother.

Rats were injected intraperitoneally at approx. 0930h (except for the newborn animals; see above) with 4μCi of D-[6-^{14}C]glucose (3–4mCi/mmol) and 16μCi of D-[6-^{3}H]glucose (200mCi/mmol)/100g body wt., in a volume of 0.5ml of 0.9% NaCl/100g body wt. The animals were killed by decapitation at 15min intervals up to 75min after injection. Samples of blood were collected and liver and skeletal-muscle samples were removed in that order for analysis. Blood and tissue samples for each time-interval were pooled from four newborn rats, from four 2-day-old animals and from two 10-day-old rats. In the experiments on the 2-day-old rats, animals from two litters had to be pooled. The infant rats were therefore divided randomly at the time of injections into groups of two or four, depending on ages.

Chemicals

[6-^{14}C]Glucose and [6-^{3}H]glucose were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). NAD^+, muscle lactate dehydrogenase (EC 1.1.1.27) (360 units/mg) and peroxidase (EC 1.11.1.7) were from Boehringer Corp. (London) Ltd. (London W.5, U.K.) and glucose oxidase (EC 1.1.3.4) (type II) was from Sigma (London) Chemical Co. Ltd. (London S.W.6, U.K.). Scintillation-grade 1,4-bis-(5-phenyloxazol-2-yl)benzene and 2,5-diphenyloxazole were obtained from Nuclear Enterprises Ltd., Edinburgh, U.K. Triton X-100 was from Lennig Chemicals Ltd., Jarrow, Co. Durham, U.K.

Determination of the specific radioactivity of plasma glucose and lactate

Rat blood was collected in ice-cold heparinized tubes and centrifuged immediately at 1000rev./min for 4min at 4°C. Plasma was removed and stored at −10°C until processed about 4h later. Samples of plasma (0.05ml) were deproteinized with 2.45ml of 0.27M-$HClO_4$, and the glucose concentration was measured by the glucose oxidase method of Krebs *et al.* (1963). Other samples of plasma (0.1ml) were deproteinized with 0.40ml of 0.27M-$HClO_4$ and the L-lactate concentration was measured by the method of Hohorst (1963).

Plasma glucose and lactate were separated by paper chromatography by using a method similar to that of Depocas (1959*a*). We confirmed the suitability and specificity of this procedure for the present work. Samples of plasma (0.05ml) were streaked (7cm) on 11cm-wide strips of Whatman no. 1 chromatography paper, and the chromatogram was developed by descending chromatography for 16h at room temperature in the solvent butan-1-ol–acetic acid–water–*n*-butyl acetate (120:24:49:9, by vol.). After drying, the chromatograms were cut into transverse strips 2cm wide, which were then eluted into scintillation vials with 1.0ml of water. The radioactivity of the samples was measured after the addition of 9.0ml of scintillation fluid (see below). Initial experiments indicated that over 98% of the radioactivity was recovered in the eluates from the strips. Only two bands of radioactivity were found on the chromatograms, one with the same mobility as glucose and the other with that of L-lactate; the glucose peak appeared at 8.0cm from the origin, and the L-lactate peak at 32.0cm from the origin.

Determination of specific radioactivity of liver and muscle glycogen

Liver and muscle samples (approx. 1g) were digested with 2.0ml of 40% (w/v) KOH at 100°C. Glycogen was purified by the method of Cowgill & Pardee (1957). The glycogen was washed three times with water; further washings were found to be free of radioactivity. The glycogen was hydrolysed to glucose with 1M-H_2SO_4 for 2h at 100°C, and samples of the hydrolysate were used for radioactivity determination. Other samples of the hydrolysate after suitable dilution were used for glucose determination by the glucose oxidase method.

Measurement of radioactivity

Aqueous samples (1.0ml) were mixed with 9.0ml of a Triton–toluene (1:2, v/v) scintillation fluid [the toluene containing 4g of 2,5-diphenyloxazole and 0.05g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l], and were counted for radioactivity in a Nuclear–Chicago model no. 725 automatic liquid-scintillation spectrometer. The counting efficiencies, and the proportion of the measured disintegrations due to ^{14}C and ^{3}H in the samples, were determined by the channels-ratio method of Hendler (1964). Results are given corrected to 100% efficiency.

Statistical calculations and analysis

The log specific radioactivity (d.p.m./μmol) of plasma glucose was calculated in each case and plotted against the time after injection of the [6-^{14}C]-glucose and [6-^{3}H]glucose; the values obtained from five or six separate experiments on animals of each age group (but three experiments on the newborn animals) were pooled, and a regression analysis was performed and tested for linearity as described by Mather (1964). The half-life of the specific radioactivity of plasma glucose was calculated from the regression coefficient. The fractional turnover rate of plasma glucose and the proportion of glucose recycled via the Cori cycle were calculated as described by Dunn *et al.* (1967), and the glucose pool and glucose space in equilibrium with plasma glucose were calculated as described by Feller *et al.* (1950).

Results

The body weights, liver weights and liver weight/body weight ratios of the rats at each of the ages studied are summarized in Table 1. At each age, the values of these parameters did not vary significantly between the groups of rats killed for sampling at each time-interval after injection.

Glucose turnover and pool size as a function of age

Table 2 shows that there was no significant change in the plasma glucose concentration after the injection of labelled glucose during the time-period studied for 2-, 10- and 30-day-old rats. The plasma glucose concentrations of the newborn rats decreased with time. There was also a significant increase

Table 1. *Body weights, liver weights and liver weight/body weight ratios of the rats at each age*

Results are expressed as means±S.E.M.

Age (days)	No. of observations	Body wt. (g)	Liver wt. (g)	100×liver wt./body wt.
Newborn	9	6.0±0.06	0.33±0.01	5.57±0.13
2	26	7.4±0.27	0.27±0.01	3.66±0.08
10	27	19.4±0.58	0.56±0.02	2.91±0.04
30	30	77.2±1.62	3.50±0.10	4.54±0.07

Table 2. *Plasma glucose concentration as a function of time after intraperitoneal injection of rats of different ages with [6-^{14}C]glucose and [6-^{3}H]glucose*

Experimental details are given in the text. Results are given as means±S.E.M. of the number of observations in parentheses.

Age of rats (days)	Plasma glucose concentration (mM) at the following times after injection of labelled glucose					Mean plasma glucose concentration (mM) over experimental period
	15min	30min	45min	60min	75min	
Newborn	6.17±0.50 (3)	—	5.49±0.20 (3)	—	4.79±1.11 (3)	—
2	4.79±0.42 (4)	5.25±0.40 (5)	4.91±0.81 (5)	5.02±0.62 (5)	4.83±0.23 (5)	4.97±0.22 (24)
10	6.45±0.53 (5)	6.50±0.38 (6)	6.77±0.35 (5)	6.64±0.38 (5)	6.06±0.49 (6)	6.47±0.18 (27)
30	7.52±0.43 (6)	6.75±0.34 (5)	7.80±0.19 (5)	7.32±0.36 (5)	7.30±0.39 (5)	7.34±0.16 (26)

($P<0.01$) in the mean plasma glucose concentration between 2 and 30 days after birth.

The regression of the log of the specific radioactivity of glucose with time was found to be linear for both [6-^{14}C]glucose and [6-^{3}H]glucose over the period from 15 to 75min after injection for 2- and 30-day-old rats, but from 15 to 60min only for 10-day-old rats.

The values for the log(glucose pool size) (Table 3) expressed per 100g body wt. and obtained by using the results for both [6-^{14}C]glucose and [6-^{3}H]-glucose were in good agreement for the 2-, 10- and 30-day-old animals, and there was no significant difference between the values found at these three ages. The size of the glucose pool was not significantly altered at any age by the amount of glucose injected, this being less than 1.0% of the pool at all the ages.

The glucose space, i.e. the volume of body water in which the glucose pool is distributed, was found to decrease with age (Table 3); in the 2-day-old rat, it may be equivalent to the total body water and may reflect the high lean-body mass after depletion of reserves.

The fractional turnover rate of the glucose pool increased with age, the values for the 30-day-old rat being significantly greater than those of both the 2- and 10-day-old animals ($P<0.01$). These age differences were minimized on calculation of the glucose turnover rate (fractional turnover rate multiplied by the glucose pool expressed in μmol/100g body wt.) because of the changes in the glucose pool size with age, but the glucose turnover rate of the 30-day-old rat was still approximately twice that of the suckling animals.

Although the values of the fractional turnover rates of the glucose pool at each age obtained by using either [6-^{14}C]glucose or [6-^{3}H]glucose did not differ significantly, the values obtained with [6-^{3}H]glucose are always greater than those obtained with [6-^{14}C]-glucose. Calculation of the proportion of glucose recycled via the Cori cycle gave results that did not differ significantly from zero, so that these results certainly indicate that less than 10% of the glucose utilized is thus recycled at all the ages studied.

The specific radioactivity of both plasma [6-^{14}C]-glucose and [6-^{3}H]glucose in the injected newborn rats did not change significantly during the experimental period, the specific radioactivity at 15, 45 and 75min after the injection of labelled glucose being 14.5±0.7, 16.0±0.3 and 12.6±0.5d.p.m./nmol of glucose for [6-^{14}C]glucose and 60.7±5.1, 64.7±1.5 and 58.3±3.8d.p.m./nmol of glucose for [6-^{3}H]-glucose respectively. Each of the above values is a mean of three observations±S.E.M. and thus a mean value for 12 newborn rats. These values correspond to a glucose pool of 570μmol of glucose/100g body

Table 3. *Effect of age on the glucose pool, the glucose space, half-life and turnover rate and the proportion of glucose recycled via the Cori cycle*

Experimental details, definition of terms and methods of calculation are described in the text. S.E.M. and S.D. values are reported where they can be calculated.

Age	2 days		10 days		30 days	
Isotope	[6-^{14}C]Glucose	[6-^{3}H]Glucose	[6-^{14}C]Glucose	[6-^{3}H]Glucose	[6-^{14}C]Glucose	[6-^{3}H]Glucose
Number of pooled results	24		27		26	
Glucose concentration ± S.E.M. (μmol/ml of plasma)	4.97 ± 0.22		6.47 ± 0.18		7.34 ± 0.16	
log[Glucose pool size (μmol/100g body wt.)] ± S.D.	2.547 ± 0.046	2.599 ± 0.065	2.464 ± 0.036	2.467 ± 0.036	2.439 ± 0.070	2.416 ± 0.086
Glucose pool size (mean) (μmol/100g body wt.)	352.0	361.9	291.0	293.1	275.0	260.8
Glucose space (ml/100g body wt.)	70.8	72.8	45.0	45.3	37.5	35.7
Fractional turnover rate ± S.D. (fraction of glucose pool/min)	0.0181 ± 0.0019	0.0183 ± 0.0037	0.0220 ± 0.0018	0.0238 ± 0.0019	0.0424 ± 0.0030	0.0465 ± 0.0037
Half-life (min)	38.28	37.86	31.51	29.13	16.36	14.92
Glucose turnover rate (μmol of glucose/min per 100g body wt.)	6.37	6.62	6.40	6.98	11.66	12.13
% recycled via Cori cycle	1.1		7.6		9.8	

wt. in the newborn rat. A precise value of the glucose space cannot be calculated in view of the changing plasma glucose concentration; the results indicate, however, that the glucose space of the newborn rat is at least as great as that of the 2-day-old and may well be equal to the total body water, 80–85 ml/100 g body wt. at birth (Spector, 1956).

Incorporation of radioactivity from glucose into plasma L-*lactate*

There was no significant change in the plasma L-lactate concentration in the 2-, 10- and 30-day-old rats during the time-period studied after injection of labelled glucose (Table 4). These plasma L-lactate concentrations at the several ages agree with those reported by Burch (1965). Because the mean plasma L-lactate concentration varied with age, the amount of ^{14}C incorporated into L-lactate is expressed as d.p.m./ml of plasma. Results for the incorporation of ^{3}H into plasma L-lactate are not reported, as at certain times after injection the radioactivity (c.p.m.) of the samples was too small to be measured accurately; this was also found to be so for the incorporation of ^{3}H into liver and muscle glycogen (see the next section). Maximum labelling of plasma L-lactate with ^{14}C occurred 15 min after the injection of labelled glucose in the 30-day-old rats (Fig. 1) and this was followed by a steady decline in the radioactivity in the lactate. For the younger rats, however, once maximum incorporation had been obtained (after 15 min for the 2- and 10-day-old, and after 45 min for the newborn rats) no further significant changes in the d.p.m./ml of plasma in the L-lactate were observed up to 75 min after the initial injection.

Incorporation of radioactivity from glucose into liver and skeletal-muscle glycogen

The concentration of liver and skeletal-muscle glycogen did not change significantly with time after injection of radioactive labelled glucose (Tables 5 and 6) and the changes in mean concentration with age are in essential agreement with those reported previously (Shelley, 1961). The concentrations of muscle glycogen given for the newborn and 2-day-old rats will be low since the samples were not completely free of bone.

There is a considerable difference in the hepatic glycogen concentration per 100 g body wt. at the various ages (Table 5), and the amount of radioactivity incorporated into hepatic glycogen from [6-^{14}C]glucose has therefore been expressed as d.p.m./100 g body wt. (Fig. 2). As the total amount of skeletal-muscle glycogen per 100 g body wt. is not known, the incorporation results are expressed as d.p.m./μmol of muscle glycogen glucose (Fig. 3). For the 30-day-old rats, the maximum incorporation of ^{14}C into liver glycogen was observed 15 min after injection of radioisotope and the decline that followed was slow, whereas with the younger animals there was an increase throughout the whole period studied; this increase was the least for the 2-day-old animals. The total amount of ^{14}C present in liver glycogen at any time for any of the animals was always less than 1% of that injected at zero time.

The specific radioactivity (in d.p.m./μmol) of muscle glycogen-glucose increased with time over the experimental period in the rats at all ages. The absolute amount of [^{14}C]glucose incorporated was greatest at all the times in the newborn and 2-day-old rats.

Discussion

When the plasma glucose concentration does not change, as in the present experiments on 2-, 10- and 30-day-old animals, then the rate of glucose production equals the rate of glucose utilization. By 2 days after birth the rate of glucose utilization appears to have risen from a value of 1–2 μmol/min per 100 g body wt. [calculated from the data of Dawkins (1963)

Table 4. *Plasma* L-*lactate concentration as a function of time after intraperitoneal injection of rats of different ages with* [6-^{14}C]*glucose and* [6-^{3}H]*glucose*

Experimental details are given in the text. Results are means ± S.E.M. of the number of observations in parentheses.

Age of rats (days)	Plasma L-lactate concentration (mM) at the following times after injection of labelled glucose					Mean plasma L-lactate concentration (mM) over experimental period
	15 min	30 min	45 min	60 min	75 min	
Newborn	11.30 ± 2.72 (3)	—	7.50 ± 0.75 (3)	—	6.27 ± 2.19 (3)	—
2	2.64 ± 1.16 (3)	2.28 ± 0.31 (3)	2.43 ± 0.50 (3)	2.12 ± 0.45 (3)	3.27 ± 0.42 (3)	2.55 ± 0.27 (15)
10	2.17 ± 0.17 (4)	2.54 ± 0.31 (3)	3.66 ± 0.96 (3)	3.12 ± 0.17 (3)	3.39 ± 0.24 (3)	2.93 ± 0.22 (16)
30	3.63 ± 1.27 (3)	4.46 ± 1.11 (3)	3.72 ± 0.40 (3)	4.20 ± 0.62 (3)	3.60 ± 0.73 (3)	3.93 ± 0.35 (15)

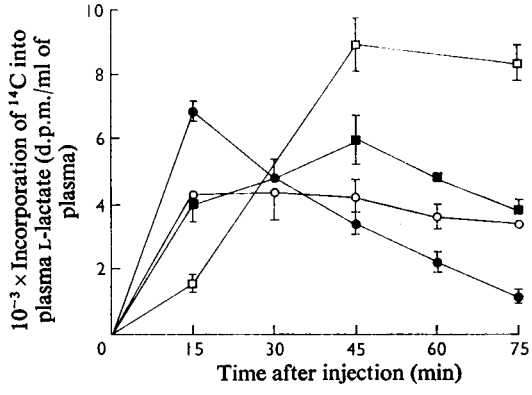

Fig. 1. *Time-course of incorporation of ^{14}C from* D-[6-^{14}C]*glucose into plasma* L-*lactate*

The D-[6-^{14}C]glucose (4μCi/100g body wt.) was administered by intraperitoneal injection at zero time; further details are given in the text. The amounts of ^{14}C incorporated are expressed as d.p.m./ml of plasma. Each point represents the mean of three or four determinations and the vertical bar shows ± S.E.M. when large enough to record. Age of rats: □, newborn; ■, 2 days old; ○, 10 days old; ●, 30 days old.

for newborn rats] to about 6.6μmol/min per 100g body wt. (Table 3). A similar rate of glucose utilization was found for the 10-day-old rat, whereas that for the 30-day-old weaned rats was approximately twice that of the younger suckling rats. This confirms the suggestion made by Hahn & Koldovský (1966, p. 76) that glucose utilization is decreased in the suckling animal.

The activities of a number of enzymes concerned with both glucose utilization and production undergo a diurnal rhythm, probably associated with the animals' eating habits (Potter *et al.*, 1966). It is possible that the glucose turnover rate will also exhibit such variations. The suckling and weaning rats appear to eat frequently throughout the 24-h day, whereas adult rats have more restricted feeding habits, usually eating during the night. The ages when adult feeding habits and the diurnal rhythms both commence and become fully established do not appear to have been investigated. At the time of killing in our experiments, the stomachs and intestines of all the 2-, 10- and 30-day-old rats contained food.

The results in Figs. 1, 2 and 3 indicate that some of the glucose utilized may be converted into liver and muscle glycogen and into lactate. In both the suckling and 30-day-old rats, less than 1% of the labelled glucose utilized was found in liver glycogen at any

Table 5. *Liver glycogen concentration as a function of time after intraperitoneal injection of rats of different ages with* [6-^{14}C]*glucose and* [6-^{3}H]*glucose*

Experimental details are given in the text. Results are means ± S.E.M. of the number of observations in parentheses.

Age of rats (days)	Liver glycogen concentration (μmol of glucose/g of liver) at the following times after injection					Mean liver glycogen concentration (μmol)	
	15 min	30 min	45 min	60 min	75 min	(per g of liver)	(per 100 g body wt.)
Newborn	295.4 ± 17.8 (3)	—	274.4 ± 17.3 (3)	—	289.5 ± 56.0 (3)	286.4 ± 17.9 (9)	1590 ± 97.1 (9)
2	80.7 ± 19.9 (5)	74.0 ± 7.4 (6)	85.4 ± 26.1 (5)	53.3 ± 14.1 (5)	62.0 ± 24.5 (5)	71.2 ± 8.1 (26)	259 ± 29.5 (26)
10	52.6 ± 9.6 (5)	72.2 ± 8.9 (6)	79.3 ± 9.6 (5)	86.2 ± 8.5 (5)	67.3 ± 7.3 (5)	71.5 ± 4.1 (26)	207 ± 12.1 (26)
30	261.6 ± 33.4 (6)	213.4 ± 32.6 (5)	251.8 ± 32.6 (5)	194.1 ± 23.7 (5)	224.3 ± 31.8 (5)	232.3 ± 13.4 (26)	1041 ± 59.7 (26)

Table 6. *Muscle glycogen concentration as a function of time after intraperitoneal injection of rats of different ages with [6-^{14}C]glucose and [6-^{3}H]glucose*

Experimental details are given in the text. Results are means±S.E.M. of the number of observations in parentheses.

Age of rats (days)	Muscle glycogen concentration (μmol of glucose/g of muscle) at the following times after injection					Mean glycogen concentration (μmol of glucose/g of muscle)
	15 min	30 min	45 min	60 min	75 min	
Newborn	37.0±1.5 (3)	—	33.3±2.8 (3)	—	31.5±0.3 (3)	33.9±1.2 (9)
2	15.8±0.8 (5)	15.3±1.4 (6)	15.1±0.9 (5)	15.5±0.7 (5)	14.0±0.8 (5)	15.1±0.4 (26)
10	18.9±1.3 (5)	21.4±1.5 (6)	17.5±2.1 (5)	22.7±1.6 (5)	18.8±1.9 (5)	20.0±0.8 (26)
30	18.9±3.0 (6)	18.4±3.8 (5)	17.7±2.7 (5)	15.7±2.1 (5)	19.4±2.0 (5)	18.1±1.2 (26)

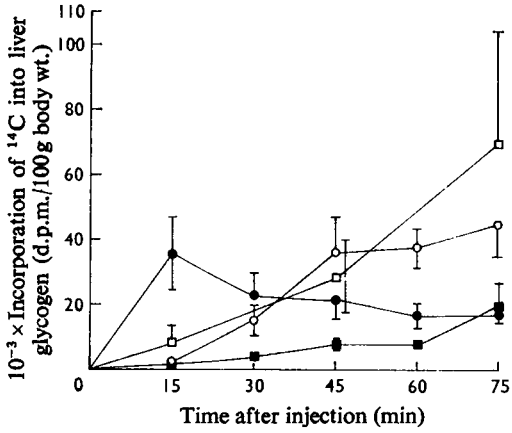

Fig. 2. *Time-course of incorporation of ^{14}C from* D-[6-^{14}C]*glucose into liver glycogen*

The D-[6-^{14}C]glucose (4μCi/100g body wt.) was administered by intraperitoneal injection at zero time; further details are given in the text. The amount of ^{14}C incorporated is expressed as d.p.m./100g of body wt. Each point represents the mean for five or six animals; the vertical bar shows ±S.E.M. when large enough to record. Age of rats: □, newborn; ■, 2 days old; ○, 10 days old; ●, 30 days old.

time, and the amount incorporated into muscle glycogen, taking a value of 1000μmol of muscle glycogen glucose/100g body wt. as the maximum at any age (Hahn & Koldovský, 1966, p. 33), was always less than 5% of that utilized. Thus, the major portion of the glucose utilized by these young rats was metabolized to non-carbohydrate compounds. Assuming that the lactate space is equal to the water space for calculation purposes, 10–12% of the label from the glucose was found in lactate 15min after the injection of [6-^{14}C]glucose in both suckling and 30-day-old rats; 75min after injection this value had decreased to less than 6%.

Because of the complication that the diet is the major source of glucose in the fed adult or weaned rat, comprising as it does approx. 60% carbohydrate, most studies of this type have used animals in the post-absorptive state. This normally involves depriving the rats of food for 5–7h before the experiment. This, however, is sufficient time to permit a decrease in both the blood glucose and hepatic glycogen concentration (Heath & Threlfall, 1968). It may also result in an increase in the activity of phosphopyruvate carboxylase, for the activity of this enzyme in the adult rat has been shown to double in less than 5h in response to various stimuli (Lardy *et al.*, 1964). Studies with adult rats in the post-absorptive state have indicated rates of glucose production ranging from 3.5 to 6.0μmol/min per 100g body wt. (Baker *et al.*, 1959; Depocas, 1959*b*; Ashby *et al.*, 1965; Dunn *et al.*, 1969). Thus more than 50% of the measured rate of glucose production (Table 3) in the fed 30-day-old rat may be due to glucose absorption from the intestine.

The contribution of dietary carbohydrate to glucose production in the suckling rat is uncertain. Assuming that the rate of glucose utilization remains constant throughout the day, a 2-day-old rat will utilize approx. 700μmol of glucose/day (calculated from the glucose turnover rate) and a 10-day-old rat approx. 2000μmol/day. The maximum proportion of this glucose that could be obtained from the diet requires a knowledge of the amount of milk the suckling rat consumes per day. Indirect evidence suggests that rats less than 1 week old consume the equivalent of approx. 105kJ (25kcal)/day per 100g body wt. and older rats 89kJ (45kcal)/day per 100g body wt. (Kennedy, 1957; Hahn & Koldovský, 1966, p. 30). As rat milk has a calorific content of 7.1kJ (1.7kcal)/g (Hahn & Koldovský, 1966, p. 33) and contains approx. 200μmol of hexose/g, mainly in the form of lactose (Dymsza *et al.*, 1964), it can be calculated that

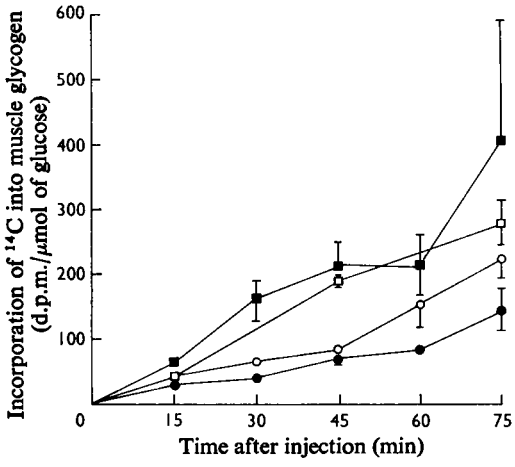

Fig. 3. *Time-course of incorporation of ^{14}C from D-[6-^{14}C]glucose into muscle glycogen*

The D-[6-^{14}C]glucose (4μCi/100g body wt.) was administered by intraperitoneal injection at zero time; further details are given in the text. The amount of ^{14}C incorporated is expressed as d.p.m./μmol of muscle glycogen glucose. Each point represents the mean of three determinations for the newborn rats and five or six for the older rats; the vertical bar shows ±S.E.M. when large enough to record. Age of rats: □, newborn; ■, 2 days old; ○, 10 days old; ●, 30 days old.

the 2-day-old rat can obtain a maximum of 30%, and a 10-day-old rat 50%, of its glucose requirement from dietary carbohydrate. This assumes that all the galactose derived from lactose is converted into glucose; some may be used for other purposes such as the formation of myelin (Moser & Karnovsky, 1959). Apart from the 24h period immediately after birth, there is a slow increase in the hepatic glycogen concentration during the neonatal period (Shelley, 1961). It follows that glycogen cannot act as a net source of blood glucose at this time. The suckling rat thus probably obtains at least 50% of its glucose requirements by gluconeogenesis.

Calculation of the rate of recycling of glucose via the Cori cycle (Table 3) showed that less than 10% of the glucose utilized was thus recycled at any age, and, because these values do not differ significantly from zero, it may be very low. Von Holt *et al.* (1961), using a different method of measurement, found that 12% of the glucose utilized was recycled via the Cori cycle in fed adult rats and more than 50% was so recycled in rats starved for 15h, whereas Dunn *et al.* (1967, 1969) found that 28% of the glucose metabolized by rats in the post-absorptive state was so recycled. The suckling rats thus differ markedly from adult rats that are in the post-absorptive or starving states. The lack of recycling in the suckling rats may reflect the fact that they are growing rapidly during a period of insufficient or limiting food supply (Widdowson & McCance, 1960) and recycling of glucose via the Cori cycle is an energy-wasteful process. It would appear that the suckling rat must be synthesizing glucose from other sources such as amino acids and glycerol. The individual contributions of these compounds to glucose production remain to be elucidated.

In the adult rat in the post-absorptive state, the value of the glucose space is 25–30ml/100g body wt. and equals the volume of the extracellular water plus plasma (Baker *et al.*, 1959; Depocas, 1959*b*; Ashby *et al.*, 1965; Dunn *et al.*, 1969). Our value for the glucose space of the 30-day-old rat is thus very similar to that of the adult in the post-absorptive state and the small difference is probably due to the different dietary status and age of the animals. Estimates of the glucose space are likely to be very sensitive to any problem caused by slow absorption of the tracer from the peritoneum. The good agreement thus noted, taken together with the fact that, of the times studied, the specific radioactivity of plasma glucose was greatest at 15min after injection, leads us to conclude that the rate of absorption is much more rapid than the glucose turnover rate (see also below). The intraperitoneal route of injection, necessitated by the small size of the very young animals, therefore appears to be acceptable.

The large glucose space in the neonatal animals (Table 3) is noteworthy. The decrease in glucose space with age can partly be explained by the decrease in plasma volume (Garcia, 1957; Constable, 1963; Miller, 1969) and the total body water with age, but other factors are probably involved.

Postnatal hypoglycaemia has been observed in several species (Shelley & Neligan, 1966). In the newborn rat, delivered by caesarean section, the blood glucose concentration decreases until 2h after birth and then begins to increase (Dawkins, 1963; Ballard & Oliver, 1963). Although there is evidence that this transient postnatal hypoglycaemia may be the factor that stimulates the onset of glycogenolysis and gluconeogenesis (Dawkins, 1963; Greengard & Dewey, 1967; Yeung & Oliver, 1968; Cake & Oliver, 1969; Greengard & Dewey, 1970), its cause remains unknown. The results presented here strongly suggest that after natural birth, hypoglycaemia results from the inability of the newborn animal to synthesize glucose, despite the fact that glucose is being catabolized. The lack of glucose turnover in the newborn rats also supports the view expressed above that the absorption of the tracer dose is completed very rapidly and that the value of the glucose pool and space at this age are not due to error caused by a relatively slow rate of glucose absorption.

The rats had been delivered naturally. The duration of the natural birth process (approx. 60min) means that the newborn animals in each group may differ in post-partum age by up to 60min. The lack of change in the specific radioactivity of the plasma glucose during the experimental period, together with the relatively small S.E.M. values (less than 10% of the mean value in each case), show that this potential complication can reasonably be disregarded in this particular case. Further investigations are clearly required to establish the precise temporal relationships of the various metabolic changes occurring during the immediate postnatal period to elucidate their possible relationships.

We are grateful to Mrs. V. A. Shakespeare and Miss C. J. Bruton for technical assistance, to Dr. J. S. Gale, Department of Genetics, for advice on the statistical problems and to the Medical Research Council and the British Nutrition Foundation Ltd. for financial assistance.

References

Ashby, M. M., Heath, D. F. & Stoner, H. B. (1965) *J. Physiol. (London)* **179**, 193–237
Baker, N., Shipley, R. A., Clark, R. E. & Incefy, G. E. (1959) *Amer. J. Physiol.* **196**, 245–252
Ballard, F. J. & Oliver, I. T. (1963) *Biochim. Biophys. Acta* **71**, 578–588
Burch, H. B. (1965) *Advan. Enzyme Regul.* **3**, 185–197
Cake, M. H. & Oliver, I. T. (1969) *Eur. J. Biochem.* **11**, 576–581
Constable, B. J. (1963) *J. Physiol. (London)* **167**, 229–238
Cowgill, R. W. & Pardee, A. B. (1957) *Experiments in Biochemical Research Techniques*, p. 158, John Wiley, New York
Dawkins, M. J. R. (1963) *Ann. N.Y. Acad. Sci.* **111**, 203–211
Depocas, F. (1959*a*) *Can. J. Biochem. Physiol.* **37**, 175–181
Depocas, F. (1959*b*) *Can. J. Biochem. Physiol.* **37**, 285–295
Drahota, Z., Hahn, P., Kleinzeller, A. & Kostolánská, A. (1964) *Biochem. J.* **93**, 61–65
Dunn, A., Chenoweth, M. & Schaeffer, L. D. (1967) *Biochemistry* **6**, 6–11
Dunn, A., Chenoweth, M. & Schaeffer, L. D. (1969) *Biochim. Biophys. Acta* **177**, 11–16
Dymsza, H. A., Czajka, D. M. & Miller, S. A. (1964) *J. Nutr.* **84**, 100–106
Feller, D. D., Stristower, E. H. & Chaikoff, I. L. (1950) *J. Biol. Chem.* **187**, 571–588
Garcia, J. F. (1957) *Amer. J. Physiol.* **190**, 19–24
Greengard, O. & Dewey, H. K. (1967) *J. Biol. Chem.* **242**, 2986–2991
Greengard, O. & Dewey, H. K. (1970) *Develop. Biol.* **21**, 452–461
Hahn, P. & Koldovský, O. (1966) *Utilisation of Nutrients during Postnatal Development*, Pergamon Press, London
Hahn, P., Koldovský, O., Krěček, J., Martínek, J. & Vacek, Z. (1961) *Somatic Stabil. Newly Born, Ciba Found. Symp., 1961*, 131
Hahn, P., Koldovský, O., Melichar, V. & Novak, M. (1963) *Biochem. Probl. Lipids, Proc. Int. Conf., 7th., 1962*, 385–390
Heath, D. F. & Threlfall, C. J. (1968) *Biochem. J.* **110**, 337–362
Hendler, R. W. (1964) *Anal. Biochem.* **7**, 110–120
Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 266, Academic Press, New York and London
Kennedy, G. C. (1957) *J. Endocrinol.* **16**, 9–17
Krebs, H. A., Bennett, D. A. H., de Gasquet, P., Gascoyne, T. & Yoshida, T. (1963) *Biochem. J.* **86**, 22–27
Lardy, H. A., Foster, D. O., Shrago, E. & Ray, P. D. (1964) *Advan. Enzyme Regul.* **2**, 39–47
Mather, K. (1964) *Statistical Analysis in Biology*, 5th. ed., chapters 8 and 9, Methuen, London
Miller, S. A. (1969) *Mammalian Protein Metab.* **3**, 26
Moser, H. & Karnovsky, M. L. (1959) *J. Biol. Chem.* **234**, 1990–1997
Page, M. A., Krebs, H. A. & Williamson, D. H. (1971) *Biochem. J.* **121**, 49–53
Potter, V. R., Gebert, R. A. & Pitot, H. C. (1966) *Advan. Enzyme Regul.* **4**, 247–265
Shelley, H. J. (1961) *Brit. Med. Bull.* **17**, 137–143
Shelley, H. J. & Neligan, G. A. (1966) *Brit. Med. Bull.* **22**, 34–39
Spector, W. S. (1956) in *Handbook of Biological Data*, p. 340, W. B. Saunders Co., London
Vernon, R. G. & Walker, D. G. (1968) *Biochem. J.* **106**, 321–329
Vernon, R. G., Eaton, S. W. & Walker, D. G. (1968) *Biochem. J.* **110**, 725–731
Von Holt, C., Schmidt, H., Feldmann, H. & Hallmann, I. (1961) *Biochem. Z.* **334**, 524–533
Widdowson, E. & McCance, R. A. (1960) *Proc. Roy. Soc. Ser. B* **152**, 188–206
Yeung, D. & Oliver, I. T. (1967) *Biochem. J.* **103**, 744–748
Yeung, D. & Oliver, I. T. (1968) *Biochemistry* **7**, 3231–3239
Zorzoli, A., Turkenkopf, I. J. & Mueller, V. L. (1969) *Biochem. J.* **111**, 181–185