Glucose Metabolism in the Newborn Rat

TEMPORAL STUDIES *IN VIVO*

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(Received 9 October 1972)

1. The concentrations of plasma D-glucose, L-lactate, free fatty acids and ketone bodies and of liver glycogen were measured in caesarian-delivered newborn rats at time-intervals up to 4 h after delivery. Glucose and lactate concentrations decreased markedly during the first hours after delivery, but there was a delay of 60–90 min before significant glycogen mobilization occurred. 2. The specific radioactivity of plasma D-glucose was measured as a function of time for up to 75 min after the intraperitoneal injection of D-[6- 14 C]glucose and D-[6- 3 H]glucose into caesarian-delivered rats at 0, 1 and 2 h after delivery. Calculations revealed that there was an appreciable rate of glucose formation at all ages studied, but immediately after delivery this was exceeded by the rate of glucose utilization. Around 2 h *post partum* the rate of glucose utilization decreased dramatically and this coincided with a reversal of the immediately postnatal hypoglycaemia. 3. The specific radioactivity of plasma L-lactate and the incorporation of 14 C into plasma D-glucose and liver glycogen was measured as a function of time after the intraperitoneal injection of L-[U- 14 C]lactate into rats immediately after delivery. The logarithm of the specific radioactivity of plasma L-[U- 14 C]lactate decreased linearly with time for at least 60 min after injection and the calculated rate of lactate utilization exceeded the rate of lactate formation. 4. 14 C incorporation into plasma D-glucose was maximal from 30–60 min after injection of L-[U- 14 C]lactate and the amount incorporated at 60 min was 23 % of that present in plasma lactate. Although 14 C was also incorporated into liver glycogen the amount was always less than 3 % of that present in plasma glucose. 5. The results are discussed in relationship to the adaptation of the newly born rat to the extra-uterine environment and the possible involvement of gluconeogenesis at this time before feeding is established.

Before birth the foetal rat receives its supply of nutrients in the form of glucose and amino acids from the maternal blood supply via the placenta (Hagerman & Vilee, 1960). After birth there is a change in the nutrient supply from that supplied through the placental circulation to milk. Rat milk is a high-fat low-carbohydrate diet (Dymsza *et al.*, 1964; Hahn & Koldovský, 1966), so that until weaning oxidation of lipid serves as the main supply of energy (Hahn & Koldovský, 1966; Taylor *et al.*, 1967; Augenfeld & Fritz, 1970) and the low intake of dietary glucose may be augmented by gluconeogenesis from non-carbohydrate sources (Vernon & Walker, 1972*a,b*; Walker, 1968, 1971; Zorzoli *et al.*, 1969; Philippidis & Ballard, 1969; Yeung & Oliver, 1967*a*; Ballard & Oliver, 1963, 1965).

The immediately newborn animal must depend on the oxidation of endogenous material until suckling begins. The rat foetus accumulates large amounts of hepatic glycogen towards the end of gestation and it has been suggested that these reserves are utilized immediately after birth to sustain the animal until feeding is established (Shelley, 1961; Dawkins,

1963*a*; Greengard & Dewey, 1970; Cake *et al.*, 1971). Nevertheless, despite this store of carbohydrate, the newborn rat undergoes a transient period of pronounced hypoglycaemia in the first few hours after birth (Dawkins, 1963*b*; Yeung & Oliver, 1968; Cake *et al.*, 1971). It has been suggested that this hypoglycaemia is the result of a delay in the establishment of the gluconeogenic pathway (Cake *et al.*, 1971), which is believed to be absent from foetal liver and to develop only after birth, in association with the postnatal rise in phosphoenolpyruvate carboxylase activity (EC 4.1.1.32) (Ballard & Hanson, 1967; Yeung & Oliver, 1967*b*; Philippidis & Ballard, 1969; Ballard, 1971).

The birth of a complete litter of rats can take at least 1 h, so that the use of naturally born pups (Vernon & Walker, 1972*a*) creates difficulties in establishing the precise sequence of metabolic changes that occur during the immediate postnatal period. In the present work rats were delivered by caesarian section on the last day of gestation within a few minutes of their littermates and changes in plasma metabolites and liver glycogen were then followed.

Glucose transfer rates and radioisotope incorporation from labelled glucose and lactate *in vivo* have been measured to establish the temporal development of gluconeogenesis and other processes in these surgically synchronized neonatal animals. Some of the results have been presented in a preliminary communication (Snell & Walker, 1972b).

Experimental

Materials

Chemicals. D-[6-¹⁴C]Glucose, L-[U-¹⁴C]lactate and D-[6-³H]glucose were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). NAD⁺, NADH, rabbit muscle lactate dehydrogenase (EC 1.1.1.27), 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) 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. Agidex was a gift from Glaxo Laboratories Ltd., Ulverston, Lancs., U.K. Palmitic acid was from Hopkin and Williams, Chadwell Heath, Essex, U.K. and Triton X-100 was from Lennig Chemicals Ltd., Jarrow, Co. Durham, U.K. Scintillation-grade 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)-benzene and 2,5-diphenyloxazole were obtained from Nuclear Enterprises Ltd., Edinburgh, U.K. Other chemicals were from British Drug Houses Ltd., Basingstoke, Hants., U.K. and were AnalaR grade when available.

Animals. The rats were an albino Wistar strain. Foetal ages were determined as previously described (Snell & Walker, 1972a), and term foetuses (on day 22 of gestation) were delivered by caesarian section after anaesthesia of the mother with an ether-air mixture. The foetuses were first delivered with the placentas intact and then promptly detached after the umbilical cords had been first tied. The animals were then maintained in an incubator at an ambient temperature of 35 ± 1°C without feeding. Animals that were to be suckled were placed with a lactating foster-mother immediately after delivery. The foster-mother was a rat who had recently finished giving birth naturally and her litter was replaced by the caesarian-delivered animals; suckling usually began between 1 and 2 h after birth.

Methods

Injections. All substances were injected intraperitoneally by using an Agla micrometer syringe (Burroughs Wellcome and Co., Beckenham, Kent, U.K.) equipped with a 30-gauge needle. Newborn rats were injected 10–15 min after delivery and other rats were injected as indicated with 4 μCi of D-[6-¹⁴C]glucose (3–4 mCi/mmol) or L-[U-¹⁴C]lactate (50 mCi/mmol) and 16 μCi of D-[6-³H]glucose (7–8 Ci/

mmol) per 100 g body wt., in 0.02–0.04 ml of 0.9% NaCl.

Blood sampling. Rats were killed at intervals after delivery, or after injection, by decapitation and blood samples were collected on squares of Parafilm containing heparin (100 international units), on ice. The blood was centrifuged immediately at 1000 rev./min for 5 min at 4°C in an MSE Minor centrifuge. Plasma was removed and stored on ice before subsequent procedures. Because of the small size of newborn rats measurements of glucose and lactate concentrations and radioactivities were made on plasma pooled from three individual animals at any time-point. Measurements of free fatty acid, acetoacetate and 3-hydroxybutyrate concentrations were also made on plasma pooled from three animals. The livers from individual rats were used for the determination of concentrations and radioactivities of liver glycogen.

Radioactivity measurements. The separation of plasma glucose and lactate and measurement of radioactivity were as described by Vernon & Walker (1972a). Immediately after exsanguination, livers were removed and weighed and then digested in 30% (w/v) KOH in a boiling-water bath. Glycogen was purified as described by Cowgill & Pardee (1957) and the radioactivity determined as previously described (Vernon & Walker, 1972a). The glycogen concentration was determined after isolation and hydrolysis with Agidex, a commercial glucoamylase preparation, as described by Vernon & Walker (1970). Glycogen-derived glucose was measured as described below.

Measurement of metabolites in plasma. Samples of plasma (0.1 ml) were deproteinized with 0.9 ml of 0.27 M-HClO₄. The glucose concentration was measured by the glucose oxidase method of Krebs *et al.* (1963) and the L-lactate concentration was measured, after neutralization of the protein-free supernatant, by the method of Hohorst (1963). Acetoacetate and 3-hydroxybutyrate concentrations were assayed in whole plasma as described by McGarry *et al.* (1970). Free fatty acid concentrations were measured in whole plasma as described by Chernick & Novak (1970) by using palmitic acid as the reference standard and including a set of standards with every group of determinations.

Radioactivity counting. Radioactivity (¹⁴C and ³H) was measured in a four-channel Phillips liquid-scintillation spectrometer; counts were corrected automatically for quenching by a built-in system employing a channels-ratio procedure and presented as d.p.m. after automatic correction to 100% efficiency.

Statistical calculations and analysis. The logarithm of specific radioactivity (d.p.m./μmol) of plasma glucose or lactate was calculated in each case and plotted against the time after injection of the [6-¹⁴C]-glucose and [6-³H]glucose or [U-¹⁴C]lactate. Similar

plots of the logarithm of plasma glucose or lactate concentration were also made. Values from separate experiments on animals at each age were pooled and an analysis of variance was performed to test for linearity and to determine the slope and intercept of the statistically best straight line through the data (Mather, 1964). The statistical estimates of the slopes and intercepts and their errors in the various age groups were compared and the significance of any differences was determined by using a *t* test (Mather, 1964). Computations were carried out on a Wang 500 desk-top computer (Wang Laboratories Inc., Tewksbury, Mass., U.S.A.) by using programs developed by Dr. B. Barnes, Department of Genetics, University of Birmingham.

Estimates of the glucose or lactate body pool sizes were obtained by calculating the extent of dilution of the specific radioactivity of glucose or lactate at the time of injection (Feller *et al.*, 1950). The distribution space, defined as the volume of body fluid in which the metabolite pool is distributed, was found by dividing the value for the pool size by the concentration of the metabolite in the plasma. Rates of formation and utilization of glucose or lactate at zero time were calculated from the slope of the plot of the logarithm of specific radioactivity against time, the pool size at the time of injection and the rate of change of the plasma glucose or lactate concentration during the experimental period as described by Wrenshall (1955).

Results

Blood metabolites after birth

Fig. 1(a) shows the detailed time-course of the changes in plasma glucose concentration in caesarian-delivered rats immediately after birth. Glucose concentrations fell rapidly over the first 60–90 min after delivery and then began to rise again as described in outline by other workers (Dawkins, 1963*b*; Yeung & Oliver, 1968; Cake *et al.*, 1971). Plasma lactate concentration was high at delivery and after a lag of about 30 min fell rapidly over the next 90 min (Fig. 1*b*) in parallel with a similar decline in hepatic lactate concentration (Ballard, 1971). Rats that were placed with a lactating foster-mother after birth showed changes essentially identical with those in animals kept in an incubator.

The concentration of free fatty acids in plasma showed no significant change over the first 90 min after delivery but thereafter began to rise gradually in starved animals over the next 90 min (Fig. 2). After 24 h starvation concentrations had fallen to low values, and in these animals (results not shown) liver glycogen stores were exhausted. Rats that were placed with a lactating foster-mother immediately after delivery showed no significant change in plasma free fatty acid concentration over the first 90 min after

birth (as for the starved animals), but the concentration rose sharply by 120 min (these animals began suckling between 75 and 105 min after delivery) and then gradually increased up to 24 h to a value about twice that observed in adult rats starved for 24 h (Fig. 2).

High acetoacetate and 3-hydroxybutyrate concentrations in the blood of suckling rats were reported by Drahota *et al.* (1964), Page *et al.* (1971) and Lockwood & Bailey (1971). Table 1 shows a more detailed study of the ketone bodies in the developing rat for the period immediately after birth. Plasma 3-hydroxybutyrate and acetoacetate concentrations at birth were similar to those observed in fed adult rats, but by 60 min these had both fallen to undetectable values (<0.030 mM) and remained at this low value for at least another 2 h in starved animals. After 24 h of starvation the plasma 3-hydroxybutyrate concentration was still so low that it could not be measured but some acetoacetate was demonstrable. Animals that

Fig. 1. Plasma (a) D-glucose and (b) L-lactate concentrations as a function of age in caesarian-delivered newborn rats

After delivery the rats were maintained without feeding in an incubator at an ambient temperature of $35 \pm 1^\circ\text{C}$. Further details are given in the text. Each point represents the mean of three to eight determinations and the vertical bar shows $\pm$ S.E.M.

were suckled by a nursing foster-mother after delivery had significant plasma 3-hydroxybutyrate and acetoacetate concentrations at 120 min that continued to increase up to 24 h, when they were respectively over three and four times the values in fed adult animals.

Mobilization of liver and carcass glycogen after birth

At birth the liver glycogen concentration was high, but there was an apparent lag period of 60–90 min before significant amounts of glycogen were mobilized and the concentrations began to fall (Fig. 3). A statistical evaluation of the more limited amount of information of Dawkins (1963*b*) and Cake *et al.* (1971) also revealed no significant fall in liver glycogen concentration over the first 2 h after birth.

The total carcass glycogen (i.e. in the whole animal after decapitation and removal of peritoneal viscera) in caesarian-delivered newborn rats was found to be $3358 \pm 94(6)$ μmol of glycogen glucose/100 g body wt. (mean $\pm$ S.E.M., with number of observations in parentheses) at the time of delivery. This quantity had fallen significantly ($P < 0.01$) to $2839 \pm 100(6)$ μmol of glycogen glucose/100 g body wt. 1 h later.

Glucose turnover rates after birth

The body and liver weights of the rats at each of the ages studied in this set of experiments are summarized in Table 2. At each age, the values of these parameters did not vary significantly between the groups of rats killed at each time-interval after injection.

In all but the 2-day-old rats the radioactive glucose was injected at a time when the plasma glucose concentration was changing rapidly (see Table 2) as previously described for normal uninjected rats (Fig. 1*a*). The plots of the logarithm of the plasma

Fig. 2. Plasma free fatty acid concentration as a function of age in newborn rats

The points represent unsuckled (●) and suckled (○) caesarian-derived newborns delivered at term, and adult male rats starved for 24 h (■). The suckled animals were placed with a lactating foster-mother immediately after delivery. The foster-mother was a rat who had just finished giving birth naturally and her litter was replaced by the caesarian-delivered animals; suckling began between 1 and 2 h after birth. The unsuckled animals were maintained without feeding in an incubator at an ambient temperature of $35 \pm 1^\circ\text{C}$. Determinations at all ages were made on animals from both the 'suckled' and 'unsuckled' groups. Free fatty acid concentrations were measured in plasma pooled from three animals as described in the text and are expressed as $\mu\text{equiv./ml}$ of plasma. Each point represents the mean of three to six determinations and the vertical bar shows $\pm$ S.E.M.

Table 1. Plasma 3-hydroxybutyrate and acetoacetate concentrations in rats after birth

Term foetuses were delivered by caesarian section and kept without feeding at 35°C (unsuckled) or placed with a lactating foster-mother immediately after delivery (suckled). Suckled animals began feeding between 1 and 2 h after birth. 3-Hydroxybutyrate and acetoacetate concentrations were measured in plasma pooled from three animals and are recorded as the means $\pm$ S.E.M. with the numbers of determinations in parentheses. Statistical significance of differences between suckled and unsuckled animals: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Age after delivery (h)	3-Hydroxybutyrate (mm)		Acetoacetate (mm)	
	Unsuckled	Suckled	Unsuckled	Suckled
0	0.103 ± 0.021 (4)		0.099 ± 0.020 (4)	
1	<0.030 (4)		<0.030 (4)	
2	<0.030 (3)	0.051 ± 0.014 (3)	<0.030 (3)	0.085 ± 0.034 (3)
3	<0.030 (4)	0.125 ± 0.003 (4)***	<0.030 (3)	0.424 ± 0.040 (4)*
24	<0.030 (4)	0.394 ± 0.056 (4)*	0.150 ± 0.070 (4)	0.536 ± 0.069 (4)**
Adult (fed)	0.110 ± 0.020 (4)		0.120 ± 0.017 (4)

Fig. 3. Liver glycogen concentration as a function of age in caesarian-delivered newborn rats

After delivery the rats were maintained without feeding in an incubator at $35 \pm 1^\circ\text{C}$. Further details are given in the text. The results are expressed as μmol of glycogen glucose/g of liver; each point represents the mean of five to nine determinations and the vertical bar shows $\pm\text{S.E.M.}$

glucose concentration against time were linear for the different sections of the postnatal glycaemic response observed at the different ages; the rates of change derived from such plots are given in Table 3. By extrapolation of these plots the glucose concentration at the time of injection was obtained and used to calculate the glucose space at different ages (Table 3).

The logarithm of the specific radioactivity of plasma glucose decreased linearly with time ($P < 0.01$ or better) for both $[6\text{-}^{14}\text{C}]$ - and $[6\text{-}^3\text{H}]$ -glucose over the period of the experiment at all the ages studied. The rate of decrease for $[6\text{-}^{14}\text{C}]$ glucose was not significantly different from that for $[6\text{-}^3\text{H}]$ glucose ($P > 0.05$) at any age except for the newborn rats (see Fig. 4), where the rate of fall in specific radioactivity of $[6\text{-}^{14}\text{C}]$ glucose was greater than that for $[6\text{-}^3\text{H}]$ glucose ($P < 0.01$) (see the Discussion section). At no age did the rate of change of $[^3\text{H}]$ glucose specific radioactivity exceed that of $[^{14}\text{C}]$ glucose specific activity (Table 3), and so there appears to be no significant recycling of glucose via the Cori cycle, a result similar to that found for later stages of development by Vernon & Walker (1972a).

The values obtained for the glucose pool size (Table 3) (expressed per 100 g body wt.) from the results for both $[6\text{-}^{14}\text{C}]$ - and $[6\text{-}^3\text{H}]$ -glucose were in good agreement for the 1 h- and 2 h-old unsuckled animals and the 2 h-old and 2-day-old suckled animals, but agreement was less good for the newborn animals. There was a significant fall in glucose pool size with age after birth, and suckling had had no apparent effect on this fall 2 h later. By 2 days after birth the glucose pool size was somewhat greater.

The glucose space increased dramatically after delivery (Table 3) and remained elevated for at least 2 h before decreasing slightly by 2 days *post partum*. The spuriously high value for the glucose space in the 1 h-old animals may reflect an uncertainty in the value of the glucose concentration at the time of injection, since it is during the time-period of this experiment that an abrupt reversal of postnatal hypoglycaemia takes place. The subsequent values in the 2 h-old rats (suckled and unsuckled) suggest a glucose space of the same order as the total body water, about 85 ml/100 g body wt. at birth (Spector, 1956), whereas the space in the immediately newborn rats corresponds more to estimates of the extracellular space (Baker *et al.*, 1959). The results indicate that there is a rapid uptake of glucose from the plasma pool and extracellular compartments into tissue cells immediately after delivery.

The rates of glucose formation at each age obtained by using either $[6\text{-}^{14}\text{C}]$ - or $[6\text{-}^3\text{H}]$ -glucose did not differ significantly except in the newborn animals, where a spuriously low value (see the Discussion section) was found with $[6\text{-}^3\text{H}]$ glucose (Table 3). The rate of glucose formation decreased slightly with age in the first 2 h after birth, but then increased by 2 days *post partum* to a value slightly lower than that found in 1 h-old rats. There was no significant difference between the values found in suckled and unsuckled 2 h-old animals.

The rate of glucose utilization at the time of delivery is very high, more than twice the value found in 2-day-old rats (Table 3) and even greater than the value of 11.7–12.1 $\mu\text{mol}/\text{min}$ per 100 g body wt. found in weaned 30-day-old rats (Vernon & Walker, 1972a). In 2 h-old animals, however, utilization had decreased dramatically and this is even more marked in the suckled animals.

Incorporation of radioactivity from glucose into plasma L-lactate

The changes of lactate concentrations in plasma with time at the various ages are shown in Table 4. After the injection of radioactive glucose into newborn, 1 h- and 2 h-old rats, maximum labelling of plasma lactate with ^{14}C was found 45 min later in the newborn and 1 h-old rats, and after 30 min in the suckled and unsuckled 2 h-old rats (Fig. 5a). Maximum labelling with ^3H occurred 30 min after injection in the newborn and 1 h-old rats, and 15 min after injection in the suckled and unsuckled 2 h-old rats (Fig. 5b). Once maximum incorporation was obtained the radioactivity/ml of plasma in lactate decreased only slightly in newborn and 1 h-old rats, but somewhat more rapidly in the suckled and unsuckled 2 h-old rats over the remainder of the experimental period. There was no significant difference in the pattern of incorporation of radioactivity between

Table 2. Plasma glucose concentrations at various times after injection of $[6-^{14}C]$ - and $[6-^3H]$ -glucose into rats at different times after delivery

Each determination was made on the plasma pooled from three animals. Body and liver weights were determined on individual animals. Results are given as the means $\pm$ S.E.M. (where appropriate) with the numbers of observations in parentheses.

	Time after injection (min)	Body wt. (g)	Liver wt. (g)	Plasma glucose concn. (μ mol/ml of plasma)							
				15	20	30	40	45	60	70	75
Injected 15 min after delivery	...	5.79 $\pm$ 0.02 (90)	0.341 $\pm$ 0.004 (90)	7.65 $\pm$ 0.21 (6)	7.22 (1)	6.34 $\pm$ 0.12 (5)	5.42 (1)	4.62 $\pm$ 0.18 (6)	3.68 $\pm$ 0.20 (5)	2.87 (1)	2.52 $\pm$ 0.14 (5)
Injected 1 h after delivery		5.47 $\pm$ 0.03 (42)	0.308 $\pm$ 0.005 (42)	3.01 (2)	2.82 (2)	2.33 (2)	2.74 (2)	2.72 (2)	3.30 (2)	—	3.70 (2)
Injected 2 h after delivery (unsuckled animals)		5.90 $\pm$ 0.01 (24)	0.310 $\pm$ 0.004 (24)	3.76 (2)	—	3.82 (2)	—	4.49 (2)	5.07 (2)	—	—
Injected 2 h after delivery (suckled animals)		5.51 $\pm$ 0.03 (24)	0.310 $\pm$ 0.005 (24)	3.65 (2)	—	4.42 (2)	—	4.68 (2)	4.74 (2)	—	—
Injected 2 days after birth (suckled animals)		7.63 $\pm$ 0.03 (12)	—	5.20 (1)	—	4.87 (1)	—	5.32 (1)	4.27 (1)*	—	—

* Mean concentration of all values measured in 2-day-old rats is 4.92 μ mol/ml of plasma.

Table 3. Glucose pool, the glucose space and the rates of formation and utilization of glucose in newborn rats

	Age ... Glucose label ...	At delivery		1h		2h (unsuckled)		2h (suckled)		2 days	
		¹⁴ C	³ H	¹⁴ C	³ H	¹⁴ C	³ H	¹⁴ C	³ H	¹⁴ C	³ H
Body wt. (mean) (g)											
Glucose concn. at zero											
time (mean) (μmol/ml)			5.79		5.47		5.90		5.51		7.63
Rate of change of glucose			10.6		3.89		3.26		3.01		4.92
concn. [log(μmol/ml)/min]			-0.0184		-0.0171*		+0.0071		+0.0128		
Glucose pool size at zero time					+0.0105				0		
(mean) (μmol/100g body wt.)	493	461		392	402	279	277	276	286	319	315
Glucose space at zero time											
(mean) (ml/100g body wt.)	46.6	43.6		101	103	85.4	84.9	91.4	94.8	64.9	64.0
Rate of change of glucose											
specific radioactivity											
[log(d.p.m./μmol)/min]											
Rate of glucose formation at											
zero time (μmol/min per											
100g body wt.)	9.65	5.43		8.64	8.29	7.17	5.93	5.79	6.03	7.88	7.39
Rate of glucose utilization at											
zero time (μmol/min per											
100g body wt.)	18.7	13.9		15.3	15.2	5.21	3.98	2.28	2.39	7.88	7.39

* The change in plasma glucose concentration with time in this case is made up of two components (see Table 2).

the suckled and unsuckled 2 h-old rats, and the maximum incorporation observed for these animals was similar to that found in the newborn rats and about twice that obtained in the 1 h-old rats.

Turnover of plasma lactate and incorporation of ¹⁴C into plasma glucose

Plasma glucose and lactate concentrations were changing markedly in the time-period studied after the intraperitoneal injection of [U-¹⁴C]lactate into immediately newborn rats (Table 5). The highest specific radioactivity of the plasma lactate was found at 15 min after the injection (Fig. 6*a*) and thereafter decreased rapidly. The decrease in the logarithm of the specific radioactivity of plasma lactate with time is shown in Fig. 7. This was linear (*P* < 0.001) and the rate of decrease was 0.0280 min⁻¹. The logarithm of the plasma lactate concentration also varied linearly

Fig. 4. Time-course of specific radioactivity of plasma glucose in newborn rats

Specific radioactivity of plasma D-glucose, expressed as log (d.p.m./μmol), is shown as a semi-logarithmic function of time after the intraperitoneal injection of (a) D-[6-¹⁴C]glucose (4 μCi/100g body wt.) and (b) D-[6-³H]glucose (16 μCi/100g body wt.) into caesarian-delivered newborn rats. Each point represents a single determination on the plasma pooled from three animals. Further details are given in the text. Various other parameters measured in this set of experiments are given in Tables 2 and 4.

Table 4. Plasma lactate concentrations at various times after injection of [6-¹⁴C]- and [6-³H]-glucose into rats at different times after delivery

Each determination was made on the plasma pooled from three animals and is recorded as the means (±s.e.m. where appropriate) with the numbers of observations in parentheses. Body and liver weights are as given in Table 2.

	Plasma lactate concn. (μmol/ml of plasma)									
	15	20	30	40	45	60	75			
Injected 15 min after delivery	11.63 ± 0.73 (5)	—	8.51 (2)	—	6.60 ± 0.29 (5)	5.50 (2)	3.84 ± 0.38 (5)			
Injected 1 h after delivery	5.44 (2)	4.30 (2)	3.40 (2)	2.58 (2)	2.31 (2)	2.28 (2)	2.30 (2)			
Injected 2 h after delivery (unsuckled animals)	2.67 (2)	—	2.52 (2)	—	2.58 (2)	2.67 (2)	—			
Injected 2 h after delivery (suckled animals)	2.29 (2)	—	2.41 (2)	—	2.68 (2)	2.72 (2)	—			
Injected 2 days after delivery (suckled animals)	1.65 (1)	—	2.61 (1)	—	2.05 (1)	2.18 (1)*	—			

* Mean concentration of all values measured in 2-day-old rats is 2.12 μmol/ml of plasma.

with time ($P<0.001$) and decreased at a rate of 0.0159 min^{-1} . By extrapolation, the lactate concentration at the time of injection was found to be $13.6\text{ }\mu\text{mol/ml}$ of plasma. From these values the lactate pool at the time of injection was calculated as $808\text{ }\mu\text{mol}/100\text{ g body wt.}$ distributed in a lactate space of $59.3\text{ ml}/100\text{ g body wt.}$ The latter value is of the same order as the glucose space in newborn rats (cf. Table 3). The calculated rate of lactate formation in newborn rats at the time of injection was $22.6\text{ }\mu\text{mol}/$

Fig. 5. Time-course of incorporation of (a) ^{14}C from D-[6- ^{14}C]glucose and (b) ^3H from D-[6- ^3H]glucose into plasma L-lactate

The D-[6- ^{14}C]glucose (4 $\mu\text{Ci}/100\text{ g}$ body wt.) and D-[6- ^3H]glucose (16 $\mu\text{Ci}/100\text{ g}$ body wt.) were administered simultaneously by intraperitoneal injection at a zero time in caesarian-delivered newborn rats at the time of delivery ($\bullet$), at 1 h after delivery ($\blacksquare$), at 2 h after delivery ($\blacktriangle$), and in delivered newborns suckled by a foster-mother, at 2 h after delivery ($\triangle$). The amounts of ^{14}C and ^3H incorporated are expressed as d.p.m./ml of plasma. Each point represents the mean of two to five determinations ($\bullet$) (vertical bars show $\pm\text{S.E.M.}$) or the mean of two determinations ($\blacksquare$, $\blacktriangle$ and $\triangle$); each determination was made on the plasma pooled from three animals. Various other parameters measured in these sets of experiments are given in Tables 2 and 4.

Table 5. Plasma glucose and lactate and liver glycogen concentrations at various times after injection of [6-¹⁴C]glucose or [6-¹⁴C]lactate and [6-³H]glucose into caesarian-delivered newborn rats

Glucose and lactate determinations were made on the plasma pooled from three animals; liver glycogen and liver and body weights were determined in individual animals. Results are given as the means $\pm$ S.E.M. with the numbers of observations in parentheses.

Incorporation of ^{14}C from [^{14}C]lactate into plasma lactate and glucose (see Figs. 6 and 7)					
	Time after injection (min)	...	15	30	40
Body wt. (g)				mean 5.77 ± 0.03 (57)	
Liver wt. (g)				mean 0.336 ± 0.004 (57)	
Plasma D-glucose (mM)			7.89 ± 0.12 (4)	6.11 ± 0.08 (4)	4.30 ± 0.11 (4)
Plasma L-lactate (mM)			10.29 ± 0.24 (4)	8.99 ± 0.07 (4)	6.66 ± 0.07 (4)
				7.56 ± 0.10 (3)	3.43 ± 0.12 (4)
					5.13 ± 0.11 (4)
Incorporation of ^{14}C and ^3H from [6- ^{14}C , ^3H]glucose or [^{14}C]lactate into liver glycogen (see Fig. 8)					
	Time after injection (min)	...	15	30	45
Body wt. (g)				mean 5.72 ± 0.02 (102)	
Liver wt. (g)				mean 0.344 ± 0.003 (102)	
Plasma D-glucose (mM)			7.74 ± 0.12 (5)	6.10 (2)	4.62 ± 0.25 (5)
Plasma L-lactate (mM)			11.29 ± 0.11 (3)	8.94 ± 0.10 (3)	7.86 ± 0.06 (3)
Liver glycogen (μmol of glucose/g of liver)			404 ± 16 (24)	413 ± 15 (15)	389 ± 12 (24)
					2.82 ± 0.03 (3)
					5.98 ± 0.08 (3)
					3.96 ± 0.17 (18)
					2.48 ± 0.15 (4)
					2.82 ± 0.04 (3)
					348 ± 22 (21)

Fig. 6. Time-course (a) of specific radioactivity of plasma L-lactate in newborn rats and (b) of incorporation of ^{14}C from L-[U- ^{14}C]lactate into plasma D-glucose in newborn rats

(a) L-[U- ^{14}C]Lactate ($4\mu\text{Ci}/100\text{g}$ body wt.) was injected intraperitoneally into caesarian-delivered newborn rats. The specific radioactivity is expressed as d.p.m./ μmol of plasma L-lactate. (b) L-[U- ^{14}C]Lactate ($4\mu\text{Ci}/100\text{g}$ 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 plasma D-glucose. Each point represents the mean of three or four determinations and the vertical bar shows $\pm\text{S.E.M.}$ Further details are given in the text. Various other parameters measured in this set of experiments are given in Table 5.

min per 100 g body wt. and the rate of utilization was $35.5\mu\text{mol}/\text{min}$ per 100 g body wt.

The specific radioactivity of plasma glucose increased to a maximum 30 min after the injection of [U- ^{14}C]lactate into newborn rats and remained constant over the next 30 min (Fig. 6b). This time-course differs from that observed with 2- or 10-day-old rats in which the specific radioactivity was maximal at 15 min and thereafter decreased rapidly (Vernon & Walker, 1972b).

Incorporation of radioactivity from glucose and lactate into liver glycogen

The concentration of liver glycogen did not change significantly ($P > 0.05$) with time during the period studied in the experiments with labelled glucose or

Fig. 7. Time-course of specific radioactivity of plasma lactate in newborn rats

Specific radioactivity of plasma L-lactate, expressed as $\log(\text{d.p.m./}\mu\text{mol})$, is presented as a semi-logarithmic function of time after the intraperitoneal injection of L-[U- ^{14}C]lactate ($4\mu\text{Ci}/100\text{g}$ body wt.) into caesarian-delivered newborn rats at delivery. Each point represents a single determination on the plasma pooled from three animals. Various other parameters measured in this set of experiments are given in Table 5.

lactate, but plasma glucose and lactate concentrations decreased in the expected fashion (Table 5). Incorporation of radioactivity from labelled glucose into liver glycogen increased throughout the whole time-period studied (Fig. 8) in a manner similar to that observed by Vernon & Walker (1972a) for naturally delivered newborn rats. A similar pattern was found for incorporation from labelled lactate. The total amount of radioactivity present in liver glycogen at any time from any of the labelled precursors was always less than 0.5% of that injected at zero time.

Discussion

Determination of recycling by using D[6- ^{14}C , ^3H]-glucose

The use of [6- ^3H]glucose in glucose turnover studies allows measurement of new glucose formation free from any error caused by recycling of glucose carbon via the Cori cycle, since resynthesis of glucose from lactate in the liver via the dicarboxylic acid shuttle involves a virtually complete loss of ^3H from C-6 of glucose (Dunn *et al.*, 1967). Further, the simultaneous use of [6- ^3H]- and [6- ^{14}C]-labelled

Fig. 8. Time-course of incorporation of ^{14}C from D-[6- ^{14}C]glucose (●) or L-[U- ^{14}C]lactate (▲) and ^3H from D-[6- ^3H]glucose (■) into liver glycogen in caesarian-delivered newborn rats

The D-[6- ^{14}C]glucose or L-[U- ^{14}C]lactate ($4\mu\text{Ci}/100\text{g}$ body wt.) and D-[6- ^3H]glucose ($16\mu\text{Ci}/100\text{g}$ body wt.) were administered by intraperitoneal injection at zero time. The amounts of ^{14}C and ^3H incorporated are expressed as d.p.m./μmol of liver glycogen glucose. Each point represents the mean of two to five determinations and the vertical bar shows $\pm\text{S.E.M.}$, when large enough to record. Various other parameters measured in these sets of experiments are recorded in Table 5.

glucose permits an estimation of the extent of recycling via the Cori cycle, since the apparent turnover of [^{14}C]glucose will be less than that of [^3H]glucose by an amount caused by recycling of the [^{14}C]glucose. This approach was adopted successfully in previous work (Vernon & Walker, 1972a) to determine the proportion of the glucose pool recycled in developing rats at different ages.

In the present experiments the turnover of [6- ^{14}C]glucose in immediately newborn rats was significantly greater than that of [6- ^3H]glucose ($P < 0.01$). Hence not only is there no evidence of recycling of glucose carbon via lactate, but also there are reactions occurring that promote incorporation of ^3H from labelled glucose into unlabelled glucogenic precursors. A possible process is conversion of [6- ^3H]glucose in the liver immediately at birth into [3- ^3H]pyruvate by glycolysis followed by oxidation of this pyruvate in the tricarboxylic acid cycle resulting in formation of ^3H -labelled NADH. Since immediately at birth the rat is in a relatively hypoxic condition, aerobic re-

oxidation of the NADH does not occur rapidly (Ballard, 1971). The reduced nucleotide could, therefore, serve as a source of reducing power in the glyceraldehyde 3-phosphate dehydrogenase reaction in glucose synthesis from precursors, thus resulting in ^3H incorporation into glucose. Rognstad & Katz (1971) showed that intramitochondrial ^3H -labelled NADH can promote tritium incorporation into glucose. This process might only apply immediately after birth because once the perinatal hypoxia has been overcome and the liver is fully oxygenated (in the first hour after birth; Ballard, 1971) intramitochondrial NADH will be rapidly reoxidized aerobically.

The negligible recycling of glucose carbon suggested by the doubly labelled glucose experiments at birth (and also at later ages) is supported by the conclusion that the principal source of plasma lactate is muscle glycogen rather than plasma glucose (see below).

Glucose transfer rates and changes in plasma metabolites

The high rate of glucose utilization immediately after birth (Table 3) may reflect the hypoxic state of the newborn rat (Ballard, 1971) and the consequent dependence on anaerobic glycolysis in the period immediately after birth. By 2 days after birth the rate of utilization has fallen to about half the value for newborn rats and this lower rate continues throughout the suckling period (Vernon & Walker, 1972a). It thus appears that in the suckling animal glucose utilization is decreased (see also Vernon & Walker, 1972a; Hahn & Koldovský, 1966) as the animal adapts to the utilization of dietary fatty acids as primary energy source. Such an adaptation has apparently not taken place in the immediately newborn animal whose metabolism is characterized by a dependence on carbohydrate metabolism for energy production, a conclusion also reached by De Meyer *et al.* (1971) on the basis of respiratory-quotient measurements, metabolic-balance studies and oxygen consumption.

As soon as the concentration of plasma free fatty acids increases (at 2 h *post partum*, see Fig. 2) there is a dramatic fall in the rate of glucose utilization (Table 3) and, in suckled rats, an increase in plasma 3-hydroxybutyrate and acetoacetate concentrations (Table 1) indicating an increased rate of hepatic fatty acid oxidation. The lipid stores of the rat are low at birth (Widdowson, 1950; Hahn & Koldovský, 1966; Hahn, 1970), and starvation for 24 h after birth results in decreased plasma free fatty acid concentrations (Fig. 2) and decreased plasma ketone-body concentrations (Table 1), presumably owing to depletion of the endogenous reserves. However, in animals allowed to suckle after birth there is a much greater increase in

the plasma free fatty acid concentration by 2h *post partum* than in unsuckled animals ($P < 0.001$) (Fig. 2) and a greater decrease in the rate of glucose utilization ($P < 0.001$) (Table 3). In suckled rats at 24h after birth plasma free fatty acid and ketone body concentrations have increased further (Fig. 2 and Table 1). These observations emphasize the importance of dietary lipid as the source of free fatty acids in the newborn rat. Fatty acids are known to inhibit glucose uptake and utilization in certain muscle tissues (heart and diaphragm) in adult rats (Garland & Randle, 1964; Garland *et al.*, 1964a,b; Randle *et al.*, 1966), and the decreased rate of glucose utilization in newborn rats 2h after delivery (Table 3) may, therefore, be causally related to the increase in the plasma free fatty acid concentration at this time. Similarly, the apparent delay in lipolysis and free fatty acid release into the circulation until 1–2h *post partum* (Fig. 2) may be related to the high plasma concentrations of glucose and lactate immediately after birth (Figs. 1a and 1b). The availability of glucose prevents free fatty acid release from adipose tissue in adult rats and depresses fatty acid utilization in muscle tissues (Randle *et al.*, 1963, 1966), and high circulating lactate concentrations also inhibit free fatty acid release from adipose tissue (Bjorntrop, 1966; Houghton *et al.*, 1971). These relationships between plasma glucose and free fatty acid concentrations in the newborn rat may be a consequence of the pronounced fall in plasma insulin concentration (Blazquez *et al.*, 1970) and the rise in growth hormone concentration (Birge *et al.*, 1967) after birth, both of which would favour lipolytic effects and a decrease in glucose utilization by muscle.

Vernon & Walker (1972a) could measure no turnover of glucose in naturally delivered newborn rats. Natural birth can take 1 h for a complete litter and such animals will have differed in age by up to 60 min within a litter at the time of injection of radioactive glucose. Since plasma samples from animals were pooled for measurements at each time-point, and considering the wide range of plasma glucose concentrations (cf. Fig. 1a), it seems likely that the values for glucose specific radioactivity would be 'averaged' ones and would not show any recognizable downward trend with time during the experiment. The lack of a significant fall in the plasma glucose concentration as a function of time in newborn rats in Table 2 of Vernon & Walker (1972a, p. 523) supports this reasoning. Such problems were eliminated in the present work by delivering the rats almost together by caesarian section, which resulted in substantial rates of glucose formation being observed (Table 3). The rates of glucose formation measured at various ages after delivery were similar in newborn, 1 h- and 2 h-old unsuckled rats but were slightly less in suckled 2 h-old animals (Table 3). Thus the dramatic changes in plasma glucose concentrations that occur after

birth appear to be due to alterations in the rate of glucose utilization rather than in the rate of glucose production.

The rates of glucose formation measured after birth could be due to gluconeogenesis *per se* and/or to hepatic glycogenolysis. Immediately after birth glycogen breakdown does not seem to be a major source of glucose formation. Measurements of hepatic glycogen concentration after delivery (Fig. 3) show a delay of between 1 and 2 h before significant mobilization occurs. Incorporation of radioactivity into glycogen from either the [^{14}C]glucose or [^3H]glucose accounted for less than 0.5% of the labelled precursors utilized at any time. Taken together with the time-course studies (Fig. 8) this suggests a low rate of synthesis of hepatic glycogen in the newborn rats with very little turnover of the glycogen pool. Any decrease in the hepatic glycogen concentration is likely, therefore, to represent the absolute rate of glycogen degradation, and the steady concentration of glycogen observed for the first 1–2 h after birth suggests that no glycogen has been mobilized. The body glucose pool decreases by $101 \mu\text{mol}/100 \text{ g}$ of body wt. during the first hour after birth (Table 3). Since the liver glycogen pool at birth is $2610 \mu\text{mol}/100 \text{ g}$ body wt. (Fig. 3), mobilization of less than 5% of this glycogen pool would be sufficient to prevent the fall in glucose concentration. This does not occur, implying that the glycogenolytic rate at birth is much less than 5% of the pool per h.

The rates of lactate turnover at birth are high, but the rate of incorporation of [$6\text{-}^{14}\text{C}$]glucose into plasma lactate at birth (Fig. 5) indicates a rate of lactate formation from glucose of $< 5 \mu\text{mol}/\text{min}$ per 100 g body wt. Since the rate of lactate formation after [$\text{U-}^{14}\text{C}$]lactate injection is calculated from the specific-radioactivity data as $22.6 \mu\text{mol}/\text{min}$ per 100 g body wt., the majority of the lactate must be derived from muscle glycogen. The fall in carcass glycogen during the first postnatal hour ($519 \mu\text{mol}$) was indeed sufficient to account for the observed rate of lactate formation ($1056 \mu\text{mol}$ of lactate/h per 100 g body wt.) after allowing for the contribution from plasma glucose. The rate of lactate utilization ($35.5 \mu\text{mol}/\text{min}$ per 100 g body wt.) exceeds its rate of formation and consequently the plasma concentration of lactate falls after birth (Fig. 1b). A significant proportion of the lactate utilized may be used for glucose synthesis and the rate of utilization is more than sufficient to account for the observed rate of glucose formation at birth. The rate of incorporation of radioactivity from lactate into glucose in the newborn rat (Fig. 6b) is only about 50% of that observed in the weaned 30-day-old rat (Vernon & Walker, 1972b), but because of the extremely large lactate pool in the newborn animal ($808 \mu\text{mol}/100 \text{ g}$ body wt.), which is about ten times greater than in the 30-day-old rat (Vernon & Walker, 1972b), the actual

rate of glucose synthesis from lactate in the newborn rat will be much greater than at 30 days. Measurements of net glucose synthesis from various precursors in liver slices of newborn rats showed lactate to be the most potent substrate (apart from fructose) for gluconeogenesis at that age (Vernon *et al.*, 1968).

The results obtained in the present work do not indicate the precise time of initiation of gluconeogenesis in the rat after birth, although it is apparent that this occurs within the first hour after delivery. The results for dilution of injected radioactive glucose (Fig. 4) were obtained over a period of 75 min after injection at the time of delivery. It is not possible to study a shorter time-period because the scatter of the results would be too great to allow differences in glucose specific radioactivity with time to be determined. The wide scatter is an inherent feature of employing individual animals to measure specific radioactivity necessitated by the small size of the rats that prevents multiple sampling of blood from a single animal. Nevertheless, inspection of the results in Figs. 4(a) and 4(b) suggests some delay in the fall of plasma glucose specific radioactivity until about 30 min after injection, and this would be consistent with the 'oxygenation' phase observed by Ballard (1971) immediately after birth which, it is suggested, is necessary before gluconeogenesis can proceed. There appears to be a discrepancy in the value for the net loss of body glucose in the first hour after birth when the fall in the estimated glucose pool size (101 $\mu\text{mol}/100\text{g}$ body wt.; Table 3) is compared with the difference between glucose utilization and glucose formation over 60 min (approx 400 $\mu\text{mol}/100\text{g}$ body wt.; Table 3). This can, in part, be reconciled if there were indeed a delay of some 30 min in the establishment of significant turnover of glucose. Ballard (1971) has shown that the hepatic lactate concentration remains constant over the first 30 min after delivery before beginning to fall, and inspection of the results in Fig. 1(b) suggests that this may also be true for the plasma lactate concentration; this would again indicate a delay in the establishment of gluconeogenesis immediately after birth.

We are grateful to Mrs. A. G. Phillips for skilled and conscientious technical assistance and to the Wellcome Trust for generous financial support.

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