Glucose Metabolism in the Newborn Rat

HORMONAL EFFECTS *IN VIVO*

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1. The concentrations of liver glycogen and plasma D-glucose were measured in caesarian-delivered newborn rats at time-intervals up to 3 h after delivery after treatment of the neonatal rats with glucagon, dibutyryl cyclic AMP, cortisol or cortisol+dibutyryl cyclic AMP. Glycogenolysis was promoted by glucagon or dibutyryl cyclic AMP in the third hour after birth but not at earlier times. Cortisol and dibutyryl cyclic AMP together (but neither agent alone) promoted glycogenolysis in the second hour after birth, but no hormone combination was effective in the first postnatal hour. 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-[6-¹⁴C]glucose and D-[6-³H]glucose into newborn rats at delivery and after treatment with glucagon or actinomycin D. Glucagon-mediated hyperglycaemia at this time was due to an increased rate of glucose formation and a decreased rate of glucose utilization. Actinomycin D prevented glucose formation and accelerated the rate of postnatal hypoglycaemia. 3. The specific radioactivity of plasma L-lactate and the incorporation of ¹⁴C into plasma D-glucose was measured as a function of time after the intraperitoneal injection of L-[U-¹⁴C]lactate into glucagon- or actinomycin D-treated rats immediately after delivery. The calculated rates of lactate formation were unchanged by either treatment, but lactate utilization was stimulated by glucagon administration. Glucagon stimulated and actinomycin D diminished ¹⁴C incorporation into plasma D-glucose. 4. The factors involved in the initiation of glycogenolysis and gluconeogenesis in the rat immediately after birth are discussed.

Immediately after birth the newborn rat utilizes its considerable body pools of glucose and lactate as energy sources until suckling begins and adaptation to the high-fat milk diet is complete (Snell & Walker, 1973). Towards the end of gestation large stores of hepatic glycogen are built up in the foetal rat (Shelley, 1961; Dawkins, 1963*a,b*; Greengard & Dewey, 1970), but there appears to be a lag of 1–2 h before significant amounts of the glycogen are mobilized and this may, in part, explain the pronounced hypoglycaemia experienced by the newborn rat shortly after parturition (Snell & Walker, 1973). Subsequent recovery from the hypoglycaemia coincides with the fall in hepatic glycogen concentration (Snell & Walker, 1973). Liver glycogen breakdown in the adult rat appears to be effected by activation of α -glucan phosphorylase (EC 2.4.1.1) by the hormone glucagon, acting via cyclic AMP (Exton *et al.*, 1971*a,b*; Hers *et al.*, 1970; Sutherland & Rall, 1958, 1960; Rall *et al.*, 1957). In the present paper we have examined the factors involved in the initiation of glycogenolysis immediately after birth, particularly the role of glucagon and cyclic AMP at this time. Previously we have reported that soon after birth the newborn rat is capable of synthesizing glucose from endogenous precursors (Snell &

Walker, 1973) and so we have also investigated the effect of glucagon on glucose-transfer rates *in vivo* after injection of [6-¹⁴C]glucose and [6-³H]glucose and on gluconeogenesis from lactate *in vivo* after injection of [U-¹⁴C]lactate. A preliminary account of some of this work has been given (Snell & Walker, 1972*a*).

Experimental

Materials

Animals. The rats were an albino Wistar strain; foetal ages were determined as previously described (Snell & Walker, 1972*b*). Term foetuses (on day 22 of gestation) were delivered surgically by caesarian section and maintained as described by Snell & Walker (1973).

Chemicals. Dibutyryl cyclic AMP (6-*N*,2'-*O*-dibutyryladenosine 3':5'-cyclic monophosphate) was from Boehringer Corp. (London) Ltd., London W.5, U.K.; glucagon was from Eli Lilly and Co. Ltd., Basingstoke, Hants., U.K.; cortisol acetate ('Hydrocortisyl') was from Roussel Laboratories Ltd., London N.W.10, U.K. and actinomycin D was from Calbiochem, London W.1, U.K. The sources of

other materials are as given previously (Snell & Walker, 1973).

Methods

Injections. Newborn rats were injected intraperitoneally 10–15 min after delivery by means of an Agla micrometer syringe (Burroughs Wellcome and Co., Beckenham, Kent, U.K.) equipped with a 30-gauge needle. L-[U- 14 C]Lactate (50 Ci/mol) or D-[6- 14 C]-glucose (3–4 Ci/mol) were injected at a dose of 4 μ Ci/100 g body wt. and D-[6- 3 H]glucose at a dose of 16 μ Ci/100 g body wt. in 0.02–0.04 ml of 0.9% NaCl/rat. The doses of glucagon, dibutyryl cyclic AMP, cortisol acetate and actinomycin D were 0.05, 0.25, 0.05 and 0.0025 mg/rat respectively, administered in a volume of 0.05 ml. Dibutyryl cyclic AMP and actinomycin D were dissolved in 0.9% NaCl, glucagon was dissolved in 1.6% glycerol–0.2% phenol, and cortisol acetate was dispersed in 0.9% NaCl with sodium carboxymethylcellulose and 0.9% benzyl alcohol.

Blood sampling. Rats were killed at intervals after delivery, or after injection, by decapitation. Blood samples were collected on squares of Parafilm containing heparin (100 i.u.) on ice. The blood was centrifuged immediately at 1000 rev./min for 5 min at 4°C and the plasma was removed and stored on ice during 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 similarly treated individual animals at any time-point.

Determination of specific radioactivities of plasma glucose and lactate. The separation of plasma glucose and lactate and measurement of radioactivity were as described by Vernon & Walker (1972). Determination of plasma glucose and lactate concentrations was as described previously (Snell & Walker, 1973).

Calculations. Statistical calculations and analysis of the data, estimates of metabolite pool sizes and the transfer rates of glucose and lactate were as described previously (Snell & Walker, 1973).

Results

Glycogen mobilization at birth

In previous work we reported a lag of 1–2 h in liver glycogen mobilization after caesarian delivery of term foetuses (Snell & Walker, 1973). The effect of glucagon or dibutyryl cyclic AMP on liver glycogen mobilization in surgically delivered newborn rats at various times after birth is shown in Table 1. Neither agent injected at birth had any significant effect on the glycogen concentration measured 1 h later. Glucagon injected at 1 h after delivery may have produced a slight fall in glycogen concentration at 2 h *post partum*, but this was statistically insignificant

Table 1. *Effect of glucagon and dibutyryl cyclic AMP on liver glycogen mobilization after birth*

Surgically delivered newborn rats were injected intraperitoneally at the time of delivery or at 1 or 2 h after delivery as indicated with 0.9% NaCl (controls), glucagon (50 μ g/rat) or dibutyryl cyclic AMP (0.25 mg/rat) [either alone or together with actinomycin D (50 μ g/100 g body wt.)] and killed 1 h after injection. Plasma glucose and liver glycogen concentrations were determined as described in the text and are given below as means $\pm$ S.E.M. with the number of determinations in parentheses. Glucose concentration was measured in plasma pooled from two rats and glycogen concentration was measured in individual rats. Statistical significance of results, compared with controls *** $P < 0.001$; * $P < 0.05$.

Treatment and age at death	Liver glycogen (μ mol of glycogen glucose/g of liver)	Concn. of plasma glucose (μ mol/ml of plasma)
Injected at delivery		
Control (1 h) (0.9% NaCl)	451 $\pm$ 26 (10)	4.34 $\pm$ 0.23 (5)
Glucagon (1 h)	442 $\pm$ 36 (8)	9.55 $\pm$ 0.29 (4)***
Dibutyryl cyclic AMP (1 h)	468 $\pm$ 18 (6)	10.57 $\pm$ 0.24 (3)***
Actinomycin D (1 h)	462 $\pm$ 15 (6)	3.26 $\pm$ 0.30 (3)*
Dibutyryl cyclic AMP + actinomycin D (1 h)	454 $\pm$ 19 (6)	3.85 $\pm$ 0.36 (3)
Injected at 1 h after delivery		
Control (2 h) (0.9% NaCl)	402 $\pm$ 20 (6)	2.58 $\pm$ 0.24 (3)
Glucagon (2 h)	386 $\pm$ 25 (6)	8.74 $\pm$ 0.22 (3)***
Injected at 2 h after delivery		
Control (3 h) (0.9% NaCl)	287 $\pm$ 9 (6)	3.12 $\pm$ 0.24 (3)
Glucagon (3 h)	145 $\pm$ 13 (6)***	9.62 $\pm$ 0.32 (3)***
Dibutyryl cyclic AMP (3 h)	131 $\pm$ 8 (4)***	11.25 $\pm$ 0.29 (2)***
Dibutyryl cyclic AMP + actinomycin D (3 h)	142 $\pm$ 15 (4)***	9.84 $\pm$ 0.41 (2)***

Table 2. *Effect of cortisol and dibutyryl cyclic AMP on liver glycogen mobilization after birth*

Surgically delivered newborn rats were injected intraperitoneally at the time of delivery or 1 h after delivery with 0.9% NaCl (controls), cortisol acetate (50 µg/rat), dibutyryl cyclic AMP (0.25 mg/rat) or cortisol+dibutyryl cyclic AMP and killed 1 h after injection. Plasma glucose concentration and liver glycogen concentration were determined as described in the text and are given below as the means $\pm$ S.E.M. with the number of determinations in parentheses. Glucose concentration was measured in plasma pooled from two animals and glycogen concentration was measured in individual rats. Statistical significance of results, compared with controls: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Treatment and age at death	Liver glycogen (µmol of glycogen glucose/g of liver)	Concn. of plasma glucose (µmol/ml of plasma)
Injected at delivery		
Control (1 h) (0.9% NaCl)	421 $\pm$ 17 (6)	3.92 $\pm$ 0.28 (3)
Cortisol (1 h)	443 $\pm$ 19 (6)	6.01 $\pm$ 0.32 (3)*
Dibutyryl cyclic AMP (1 h)	428 $\pm$ 19 (4)	10.42 $\pm$ 0.47 (2)**
Cortisol+dibutyryl cyclic AMP (1 h)	408 $\pm$ 20 (6)	10.36 $\pm$ 0.36 (3)***
Injected 1 h after delivery		
Control (2 h) (0.9% NaCl)	409 $\pm$ 29 (6)	2.61 $\pm$ 0.23 (3)
Cortisol (2 h)	421 $\pm$ 18 (6)	3.42 $\pm$ 0.24 (3)
Dibutyryl cyclic AMP (2 h)	398 $\pm$ 26 (4)	10.62 $\pm$ 0.34 (2)**
Cortisol+dibutyryl cyclic AMP (2 h)	97 $\pm$ 15 (6)***	13.16 $\pm$ 0.29 (3)***

($P > 0.05$). Glucagon or dibutyryl cyclic AMP injected at 2 h after delivery, however, both produced a significant and marked fall in glycogen concentration by 3 h *post partum* ($P < 0.001$). Actinomycin D administered simultaneously with dibutyryl cyclic AMP was without effect at birth, and at 2 h after delivery the inhibitor did not prevent the glycogenolytic action of dibutyryl cyclic AMP.

Despite the lack of effect of glucagon or dibutyryl cyclic AMP on liver glycogen mobilization in the first 2 h after delivery, plasma glucose concentrations were markedly elevated by each agent ($P < 0.001$) (Table 1). Actinomycin D administered simultaneously with dibutyryl cyclic AMP at birth blocked the increase in plasma glucose by the cyclic nucleotide ($P < 0.001$). At 2 h after delivery glucagon or dibutyryl cyclic AMP both markedly elevated plasma glucose concentrations ($P < 0.001$) but, in contrast, actinomycin D had no effect on this stimulation by dibutyryl cyclic AMP.

There is some evidence for the involvement of adrenal corticosteroids in hormonally induced glycogenolysis in adult rats; adrenalectomy blocks the stimulation of α -glucan phosphorylase by exogenous adrenaline or cyclic AMP in rat liver (Schaeffer *et al.*, 1969a), skeletal muscle (Schaeffer *et al.*, 1969b) and heart muscle (Miller *et al.*, 1971). The effect of cortisol on the glycogenolytic response to dibutyryl cyclic AMP at various times after delivery is shown in Table 2. Cortisol and dibutyryl cyclic AMP, injected separately or together at birth, had no significant effect on liver glycogen concentration measured 1 h later. Similarly, neither agent injected alone at 1 h *post partum* had any effect on the glycogen concentra-

Fig. 1. *Effect of glucagon or actinomycin D on plasma glucose concentration in newborn rats*

Caesarian-delivered newborn rats were injected intraperitoneally at delivery with [6-¹⁴C]glucose (4 µCi/100 g body wt.) and [6-³H]glucose (16 µCi/100 g body wt.) either alone (●) or simultaneously with actinomycin D (■) (50 µg/100 g body wt.) or glucagon (▲) (50 µg/rat). The animals were then kept at 35°C without feeding and killed at 15 min intervals. Glucose was determined on the plasma pooled from three animals as described in the text. Each point represents the mean of two to six determinations and the vertical bar shows $\pm$ S.E.M.

tion measured at 2 h, but when cortisol and dibutyryl cyclic AMP were injected together at 1 h a marked decrease in glycogen concentration had occurred by

2 h ($P < 0.001$). Dibutyl cyclic AMP administration at either age markedly elevated plasma glucose concentrations ($P < 0.001$). Cortisol alone had a less marked but significant effect on glucose concentration ($P < 0.001$) in rats at 1 h, but not at 2 h ($P > 0.05$). Cortisol and dibutyl cyclic AMP injected together at 2 h gave a greater increase in plasma glucose concentration than dibutyl cyclic AMP alone at this time ($P < 0.05$).

Effect of glucagon and actinomycin D on glucose-transfer rates

The effects of glucagon and actinomycin D on the rates of glucose formation and utilization were investigated after a single tracer injection of [6- 14 C]-glucose and [6- 3 H]glucose together. The mean body weight of the rats in this set of experiments was 5.90 ± 0.01 g (117) and, for each experimental situation, the body weight did not vary significantly between the groups of rats killed at each time-interval after injection. The plasma glucose concentration at various times after the injection of radioactive glucose, either alone or simultaneously with glucagon or actinomycin D, is shown in Fig. 1. Glucose concentration decreased with time in rats injected with label-

led glucose alone or together with actinomycin D but remained relatively constant in glucagon-treated rats. Although glucose concentration at the time of injection (by statistical extrapolation to zero time) was not significantly different in rats injected with glucagon, actinomycin D or 0.9% NaCl ($P > 0.05$), the actinomycin D treatment significantly accelerated the development of hypoglycaemia in newborn rats as compared with 0.9% NaCl-injected controls ($P < 0.001$) (Fig. 1). The results, plotted as the logarithm of the plasma glucose concentration with time, showed good linearity ($P < 0.01$) for rats injected with the labelled glucose alone or together with actinomycin D, and the rates of change derived from these plots are given in Table 3. By extrapolation of the plots the glucose concentration at the time of injection was obtained and used to calculate the glucose space (Table 3). For glucagon-injected rats, which showed no significant fall in plasma glucose concentration (Fig. 1), the mean value was used to calculate the glucose space (Table 3).

The logarithm of the specific radioactivity of plasma glucose decreased linearly with time for both [6- 14 C]- and [6- 3 H]-glucose ($P < 0.001$) over the period of the experiment in the rats injected with glucagon. The rate of decrease for [6- 14 C]glucose

Table 3. *Effect of actinomycin D and glucagon on the glucose pool, the glucose space and the rates of formation and utilization of glucose in caesarian-delivered newborn rats*

The results were calculated as described in the text from the plasma glucose specific-radioactivity decay results after the intraperitoneal injection of [6- 14 C]glucose ($4 \mu\text{Ci}/100 \text{ g body wt.}$) and [6- 3 H]glucose either alone or simultaneously with actinomycin D ($50 \mu\text{g}/100 \text{ g body wt.}$) or glucagon ($50 \mu\text{g}/\text{rat}$) into caesarian-delivered newborn rats at delivery. Each specific-radioactivity measurement was made on the plasma pooled from three animals. Statistically significant differences from untreated animals: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Treatment ...	Untreated†		Actinomycin D		Glucagon	
Glucose label ...	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H
No. of pooled results ...	19		10		10	
Glucose concn. at zero time $\pm$ S.E.M. ($\mu\text{mol}/\text{ml}$ of plasma)	10.6 ± 0.33		10.4 ± 0.26		10.2 ± 0.38	
Rate of change of glucose concn. [log($\mu\text{mol}/\text{ml}$)/min]	-0.0184		-0.0243**		0***	
Glucose pool at zero time (mean) ($\mu\text{mol}/100 \text{ g body wt.}$)	493	461	481	454	536*	518*
Glucose space at zero time (mean) (ml/100 g body wt.)	46.6	43.6	46.3	43.7	52.6*	50.7*
Rate of change of glucose specific radioactivity [log(d.p.m./ μmol)/min]	0.0196	0.0118	0***	0***	0.0220*	0.0159*
Rate of glucose formation ($\mu\text{mol}/\text{min}$ per 100 g body wt.)	9.65	5.43	0***	0***	11.9*	8.21*
Rate of glucose utilization ($\mu\text{mol}/\text{min}$ per 100 g body wt.)	18.7	13.9	11.7**	11.0**	11.9**	8.21**

† Taken from Snell & Walker (1973).

was significantly greater than that for $[6\text{-}^3\text{H}]\text{glucose}$ ($P < 0.01$), as previously observed in normal rats at birth (Snell & Walker, 1973). In animals injected with actinomycin D at delivery, there was no significant change ($P > 0.05$) in the logarithm of the specific radioactivity of plasma glucose with time for either $[6\text{-}^{14}\text{C}]$ - or $[6\text{-}^3\text{H}]\text{-glucose}$ over the period of the experiment.

The values for the glucose pool size (Table 3) expressed per 100 g body wt., and obtained by using the results for both $[6\text{-}^{14}\text{C}]$ - and $[6\text{-}^3\text{H}]\text{-glucose}$, were in fairly good agreement for each of the experimental

groups. The pool size in the glucagon-treated rats was slightly higher than that in the untreated controls ($P < 0.05$), as was the glucose space ($P < 0.05$).

The rate of glucose formation was significantly increased ($P < 0.05$) in rats injected with glucagon at birth, but even more pronounced was the effect on glucose utilization, which was significantly decreased ($P < 0.01$) (Table 3). Both of these effects will contribute to the observed stabilization of plasma glucose concentrations in newborn rats injected with glucagon (Fig. 1). Actinomycin D injection completely prevented glucose formation ($P < 0.001$) in rats at birth and also decreased the rate of glucose utilization ($P < 0.01$) (Table 3).

Effect of glucagon and actinomycin D on lactate turnover and incorporation into plasma glucose

The mean body weight of the rats in this set of experiments was $5.91 \pm 0.02\text{g}$ (96). For each of the experimental situations, the weight did not vary significantly between the groups of rats killed at each time-interval after injection. The decrease in the logarithm of the concentration of plasma lactate with time (Fig. 2) was linear in each of the experimental situations ($P < 0.01$ or better) and the rates of change are recorded in Table 4. The decrease in plasma lactate concentration in rats injected with glucagon was significantly greater than in untreated animals ($P < 0.01$) (Fig. 2, Table 4). The lactate pool and lactate space in the glucagon-treated rats were somewhat greater than in untreated animals ($P < 0.05$), but were not significantly different ($P > 0.05$) in the animals injected with actinomycin D (Table 4).

After the injection of the tracer dose of $[\text{U}\text{-}^{14}\text{C}]\text{-lactate}$ into newborn rats, the logarithm of the specific radioactivity of plasma lactate decreased linearly with time ($P < 0.001$) over the period of the experiment in each of the experimental situations. The rate of lactate formation in animals treated with glucagon or actinomycin D was not significantly different from that in untreated rats, but the rate of lactate utilization was stimulated significantly in the glucagon-treated animals ($P < 0.01$) (Table 4).

The incorporation of ^{14}C into plasma glucose after $[\text{U}\text{-}^{14}\text{C}]\text{-lactate}$ injection into newborn rats in the different experimental situations is shown in Fig. 3. Glucagon treatment markedly stimulated incorporation into plasma glucose, whereas actinomycin D decreased the lactate incorporation.

Discussion

The suggestion that there is a delay in hepatic glycogenolysis in the immediately newborn rat (Snell & Walker, 1973) is strengthened by the present results. The glycogenolytic agents glucagon or dibutyryl cyclic AMP resulted in a pronounced mobilization of

Fig. 2. Effect of (a) glucagon and (b) actinomycin D on plasma lactate concentration in newborn rats

Caesarian-delivered newborn rats were injected intraperitoneally at delivery with $[\text{U}\text{-}^{14}\text{C}]\text{-lactate}$ ($4\mu\text{Ci}/100\text{g}$ body wt.) either alone ($\bullet$) or simultaneously with actinomycin D ($\blacksquare$) ($50\mu\text{g}/100\text{g}$ body wt.) or glucagon ($\blacktriangle$) ($50\mu\text{g}/\text{rat}$). After delivery rats were kept at 35°C without feeding and killed at 15 min intervals. Lactate concentrations were determined on the plasma pooled from three animals as described in the text and are expressed as $\log(\mu\text{mol}/\text{ml}$ of plasma) and shown as a function of time. Each point represents the mean of two to four determinations. Errors are not shown for the sake of clarity, but the linear regression of each line was significant ($P < 0.01$ or better).

Table 4. *Effect of actinomycin D and glucagon on the lactate pool, the lactate space and the rates of formation and utilization of lactate in newborn rats*

The results were calculated as described in the text from the plasma lactate specific-radioactivity decay results after the intraperitoneal injection of [U- 14 C]lactate (4 μ Ci/100g body wt.) either alone or simultaneously with actinomycin D (50 μ g/100g body wt.) or glucagon (50 μ g/rat) into caesarian-delivered newborn rats at delivery. Each specific-radioactivity measurement was made on the plasma pooled from three animals. Statistically significant difference from untreated animals: ** $P < 0.01$; * $\pm < 0.05$.

Treatment ...	Untreated†	Actinomycin D	Glucagon
No. of pooled results ...	16	8	8
Lactate concn. at zero time $\pm$ S.E.M. (μ mol/ml of plasma)	13.6 $\pm$ 0.09	12.0 $\pm$ 0.27	13.5 $\pm$ 0.16
Rate of change of lactate concn. [log(μ mol/ml)/min]	-0.0159	-0.0146	-0.0190**
Lactate pool at zero time (mean) (μ mol/100g body wt.)	808	810	950*
Lactate space at zero time (mean) (ml/100g body wt.)	59.3	69.0	70.4*
Rate of change of lactate specific radioactivity [log(d.p.m./ μ mol)/min]	0.0280	0.0246	0.0210*
Rate of lactate formation (μ mol/min per 100g body wt.)	22.6	19.9	20.0
Rate of lactate utilization (μ mol/min per 100g body wt.)	35.5	31.7	42.0**

† Taken from Snell & Walker (1973).

Fig. 3. *Time-course of incorporation of 14 C from L-[U- 14 C]lactate into plasma glucose in newborn rats treated with glucagon or actinomycin D*

L-[U- 14 C]Lactate (4 μ Ci/100g body wt.) was injected intraperitoneally alone (●) or together with glucagon (▲) (50 μ g/rat) or actinomycin D (■) (50 μ g/100g body wt.) into caesarian-delivered newborn rats at birth. The specific radioactivity is expressed as d.p.m./ μ mol of plasma D-glucose. Each point represents the mean of three determinations (vertical bars show $\pm$ S.E.M.) and each determination was made on plasma pooled from three animals.

liver glycogen when injected at 2h after delivery, but when injected at the time of delivery or at 1h *post partum* there was no change in glycogen concentra-

tions (Table 1). An apparent block in hepatic glycogenolysis in late foetal rats *in utero* after maternal starvation was also reported by Goodner & Thompson (1967), and Gennser *et al.* (1971) found a similar situation in the human foetus at mid-gestation during acute intra-uterine asphyxia. On the other hand, Greengard & Dewey (1970) and Hunter (1969) reported a pronounced glycogenolytic response to an intra-uterine injection of glucagon in foetal rats in late gestation.

The dibutyl analogue of cyclic AMP exactly mimicked the observed effects of glucagon on hepatic glycogenolysis and plasma glucose concentrations (Table 1). The inability of glucagon to promote liver glycogen breakdown in the first 2h after delivery does not therefore appear to be caused by a lack of effect of the hormone on cyclic AMP production. Indeed measurements *in vitro* of the activation of adenylate cyclase (which catalyses cyclic AMP formation) by glucagon at birth, as well as the basal activity of the enzyme at that time, indicate properties very similar to those of the enzyme in adult liver (Hommes & Berre, 1971; Bär & Hahn, 1971).

Hepatic glycogen mobilization in adult rats is effected by activation of α -glucan phosphorylase (Sutherland & Rall, 1960; Hers *et al.*, 1970) and this enzyme is present in rat liver at birth at about the same activity as in the adult (Dawkins, 1963a). Cake & Oliver (1969) reported a stimulation of phosphorylase activity at 2h *post partum* compared with the activity at delivery, whereas immediately at birth a

deficiency in phosphorylase activation by glucagon or adrenaline *in vitro* was found (Dawkins, 1963a). Similarly, no change in phosphorylase activity was observed during asphyxia in the human foetal liver, nor could phosphorylase activation by adrenaline be demonstrated *in vitro* (Gennser *et al.*, 1971).

The high plasma glucose concentrations in the newborn rat immediately at birth (Snell & Walker, 1973) may be a contributory factor towards the apparent lack of phosphorylase activation at this time. Glucose inhibits phosphorylase activity *in vitro* (Buschiazzo *et al.*, 1970; Maddaiah & Madsen, 1966; Hers *et al.*, 1970; de Wulf, 1971; Bailey & Whelan, 1972) and hyperglycaemia can cause a rapid inactivation of phosphorylase in adult rat liver (Glinsmann *et al.*, 1970). Adrenalectomy is also known to block the stimulation of phosphorylase activity and glycogenolysis by cyclic AMP in adult rat liver *in vivo* (Schaeffer *et al.*, 1969a) and *in vitro* (Exton *et al.*, 1972), and adrenalectomy at birth caused a 30% decrease in the normal activation of phosphorylase at 3 h *post partum* (Cake & Oliver, 1969). Table 2 shows that cortisol injected with dibutyl cyclic AMP at delivery had no effect on liver glycogen concentrations, but injected together at 1 h *post partum* these agents promoted a marked glycogenolysis. It may be that a relative adrenal corticosteroid insufficiency at birth contributes in part to the delay in glycogen mobilization in the newborn rat. Plasma corticosterone concentrations show a prenatal peak at 18–20 days gestation and then decrease to low values on the last 2 days of gestation and in the immediately newborn rat, before rising again to reach values similar to the prenatal peak value at 2 h *post partum* (Holt & Oliver, 1968). Schapiro *et al.* (1962) and Levine *et al.* (1967) have shown that in newborn rats, in contrast with adults, corticosterone concentrations do not rise in response to stress.

Despite the lack of effect of glucagon or dibutyl cyclic AMP on liver glycogen concentrations at birth, plasma glucose concentrations were elevated by these agents (Table 1, Fig. 1). Estimates of rates of glucose formation and utilization after glucagon treatment revealed an enhancement of glucose formation and a decrease in glucose utilization (Table 3). The high rate of glucose utilization in the untreated newborn animal (Table 3; see also Snell & Walker, 1973) is probably due to the high concentration of plasma insulin at this time (Blazquez *et al.*, 1970); glucagon may antagonize the effects of insulin in some way so as to decrease glucose utilization.

The increased rate of glucose formation observed after glucagon injection into newborn rats (Table 3) is accompanied by a simultaneous increase in the rate of lactate utilization and in the rate of fall of plasma lactate concentrations with time (Table 4). This indicates that glucagon must be acting to enhance the conversion of lactate into glucose, a suggestion

that is substantiated by the observation of an increased incorporation of ^{14}C from [^{14}C]lactate into plasma glucose in glucagon-treated, as compared with untreated, newborn animals (Fig. 3). An increased incorporation of [^{14}C]pyruvate into glycogen in liver slices and *in vivo* after glucagon injection into 21-day-old rat foetuses has been observed by Philippidis & Ballard (1970) and this was accompanied by a marked fall in the hepatic lactate concentration. The effects of exogenous glucagon on metabolism of glucose and lactate observed in the present study may be of physiological significance with regard to the normally developing newborn rat, since plasma glucagon concentrations have been shown by Girard *et al.* (1972) to be elevated maximally at 30 min after birth.

The elevation of plasma glucose concentrations by dibutyl cyclic AMP in the first hour after birth was blocked by the simultaneous administration of actinomycin D (Table 1). The rise in plasma glucose concentration (Fig. 1) and the increased rate of glucose formation (Table 3; Fig. 3) after glucagon treatment at this time probably therefore requires the production of RNA and the induced synthesis of one or more enzymes involved in gluconeogenesis. Although it is not possible to identify unequivocally the entity that is involved in the increased rate of gluconeogenesis, it is generally accepted that phosphopyruvate carboxylase (EC 4.1.1.32) is the rate-limiting enzyme in this pathway at birth (Ballard & Hanson, 1967; Yeung & Oliver, 1967; Philippidis & Ballard, 1969; Ballard, 1971). Glucagon or dibutyl cyclic AMP markedly stimulate phosphopyruvate carboxylase activity *in utero*, in the immediately newborn rat and in foetal liver explants (Yeung *et al.*, 1967; Yeung & Oliver, 1968a,b; Cake *et al.*, 1971; Philippidis & Ballard, 1970; Wicks, 1969) and this induction is blocked by actinomycin D and by inhibitors of protein synthesis.

The lack of effect of actinomycin D on the dibutyl cyclic AMP-stimulated increase in plasma glucose concentrations in the third hour after delivery (Table 1) is consistent with the suggestion that the hyperglycaemia at this time is due primarily to stimulation of hepatic glycogenolysis (as judged by the decrease in liver glycogen concentrations, Table 1), since the increase in phosphorylase activity, which is presumed to be responsible for the glycogen breakdown, is an activation phenomenon that is insensitive to actinomycin D or puromycin (Sutherland & Rall, 1960; Cake & Oliver, 1969).

Actinomycin D also blocked the natural development of gluconeogenesis during the first hour after birth on the basis of measurements of glucose formation (Table 3) and of ^{14}C incorporation from [^{14}C]lactate into plasma glucose (Fig. 3). One consequence of this is to accelerate the development of hypoglycaemia immediately after delivery (Fig. 1). This effect of actinomycin D suggests that the normal

development of gluconeogenesis in the immediately newborn rat is dependent at least on the synthesis of RNA and protein. Inhibition of the synthesis of phosphopyruvate carboxylase by actinomycin D is likely, since it has been shown by immunochemical-precipitation methods that the rate of synthesis of this enzyme increases fivefold within the first 2h after delivery (Philippidis *et al.*, 1972), and both actinomycin D and puromycin inhibit the natural rise in phosphopyruvate carboxylase activity that occurs immediately after delivery (Yeung & Oliver, 1968b; Cake *et al.*, 1971). The interpretation of the results obtained with actinomycin D must, of course, be viewed with some caution in the absence of a complete understanding of the action of the inhibitor in this situation.

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