Glycogen metabolism in the liver of the neonatal *gsd/gsd* and control (*GSD/GSD*) rat

Dallas G. CLARK, Sally D. NEVILLE, Menno BRINKMAN and Owen H. FILSELL
Division of Human Nutrition, C.S.I.R.O., Kintore Avenue, Adelaide, South Australia 5000, Australia

(Received 17 August 1981/Accepted 13 November 1981)

1. The metabolism of hepatic glycogen, labelled with [6-³H]glucose at day 19.5 of gestation and with ¹⁴C from [U-¹⁴C]galactose at delivery, was followed for 10 h in food-deprived *gsd/gsd* and control (*GSD/GSD*) neonatal rats. 2. In the affected pups glycogen was maintained at 12% (w/w) and there was no loss of incorporated radioactivity. 3. The ³H and ¹⁴C in glycogen from the controls were both decreased by 80%, but ¹⁴C was removed at 0–5 h and [6-³H]glucose at 5–10 h. 4. Blood glucose concentrations in the unaffected neonatal rats fell from 5.3 mM at 20 min to 1.7 mM after 10 h. In the *gsd/gsd* pups blood glucose concentration was decreased from 2 mM at birth to 0.3 mM at 2.5 h; it was maintained at 0.8 mM between 5 and 10 h. 5. In neonatal rats that had been dead for 10 h, hepatic glycogen was decreased by 34% in the controls and by 22% in the *gsd/gsd* pups. These results demonstrate that liver from the affected rats contains glycogenolytic activity, but that it is not expressed in living tissue.

Glycogen reserves in hepatic tissue of the developing rat foetus are laid down during the last quarter of gestation (Shelley, 1961). The rapid accumulation of this carbohydrate from day 18 to day 21 coincides, not only with an increase in the total and the active form of glycogen synthase (EC 2.4.1.11), but also with an increase in total glycogen phosphorylase (EC 2.4.1.1) (Devos & Hers, 1974; Watts & Gain, 1976; Bashan *et al.*, 1979). At birth there is a rapid mobilization of liver glycogen: it is presumed that this serves to prevent hypoglycaemia until suckling and the onset of gluconeogenesis in the neonatal liver (Dawkins, 1963; Snell & Walker, 1973; Devos & Hers, 1974). During this period, as with the last 3 days of gestation, there are no substantial changes in the activities of either glycogen phosphorylase *a* or glycogen synthase *a* (Watts & Gain, 1976). This suggests that a futile cycle between glucose 1-phosphate and glycogen might occur in the liver of the late-term foetus and the neonatal rat pup. Indirect evidence for the occurrence of this cycle in hepatic tissue from the foetal rat has been presented by Goldwater & Stetten (1947), Watts & Gain (1976) and Gilbert & Bourbon (1978). It should be noted, however, that Devos & Hers (1974) have demonstrated that rodent glycogen phosphorylase is activated by anaesthesia and by stress. They have also shown that there was no detectable loss of ³H from glycogen, prelabelled with [6-³H]glucose at day 19.5 of gestation, until after parturition (Devos &

Hers, 1974, 1980a). These workers have concluded that glycogen phosphorylase *a* activity is not expressed in the foetal rat liver.

In addition to the phosphorolytic degradation of glycogen, hepatic tissue from most animals contains a second glycogenolytic mechanism. This is lysosomal and involves the hydrolytic enzyme acid α -glucosidase (EC 3.2.1.20) (Lejeune *et al.*, 1963). A deficiency of this enzyme results in a very severe form of glycogen-storage disease, Pompe's disease, which is characterized by the accumulation of glycogen within autophagic vacuoles (Hers & Barsy, 1973). Similar structures have also been observed in liver from neonatal mice (Jezequel *et al.*, 1965) and rats (Phillips *et al.*, 1967). More recent investigations have indicated that these vacuoles, glycogenosomes, might play a major role in glycogen mobilization in the neonatal rodent liver (Devos & Hers, 1980a; Iwamasa *et al.*, 1980).

We have previously described an inbred strain of rat with a non-lethal form of glycogen-storage disease (Clark *et al.*, 1980; Malthus *et al.*, 1980). The defect in this rat, a deficiency of hepatic phosphorylase *b* kinase (EC 2.7.1.38), is transmitted as Mendelian autosomal recessive (Malthus *et al.*, 1980). The lack of this enzyme prevents the degradation of the large glycogen reserves even during extended periods of food deprivation (Clark *et al.*, 1980; Malthus *et al.*, 1980). Comparative investigations of glycogen metabolism in liver from

pre-term and post-term phosphorylase kinase-deficient (*gsd/gsd*) and control (*GSD/GSD*) rat pups should indicate the role of both phosphorylase α and α -glucosidase in the perinatal metabolism of this polysaccharide.

Materials and methods

Radiochemicals

[6-³H]Glucose and [U-¹⁴C]galactose were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Enzymes

Amyloglucosidase, from *Rhizopus*, was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Glucose oxidase and peroxidase were procured, as a commercial kit, from Boehringer Mannheim Pty. Ltd., Mount Waverley, Vic., Australia. Lactate dehydrogenase was from the same source.

Animals

The animals used in this investigation are inbred substrains of the NZR/Gd line (Goodall *et al.*, 1975; Malthus & Clark, 1977). The mutation responsible for the rodent glycogen-storage disease has been designated *gsd* (Clark *et al.*, 1980). As this condition is transmitted as an autosomal recessive trait, affected animals are designated *gsd/gsd*. Unaffected littermates, with normal glycogen concentrations after food deprivation, have been inbred for over 4 years without producing any affected (*gsd/gsd*) offspring. By convention these animals are designated *GSD/GSD*. The two strains of rats (*gsd/gsd* and *GSD/GSD*) were maintained in caesarian-originated specific-pathogen-free colonies at the Division of Human Nutrition and fed on a standard diet *ad libitum*.

Handling of animals

Female rats (200–250 g body wt.) either *gsd/gsd* or *GSD/GSD* were caged with the appropriate bucks on the same night (3:00 p.m. to 9:00 a.m.) each week. The pregnant does (seven *gsd/gsd* and seven *GSD/GSD*) received two subcutaneous injections (0.3 ml each) of trace amounts of [6-³H]glucose (10 μ Ci each) at a 1 h interval on day 19.5 of gestation (Devos & Hers, 1974), and the foetuses were delivered by caesarian section under ether anaesthesia at term (day 22). The neonatal rats that were not used immediately received a single intraperitoneal injection (5 μ l) of [U-¹⁴C]galactose (1 μ Ci) and were placed in a humid atmosphere at 37°C. At the appropriate time intervals the pups were weighed, then killed by decapitation, and blood (50 μ l) was collected from the neck and deproteinized with 0.45 ml of 95% (v/v) ethanol. The livers of the neonatal rats were rapidly removed, blotted dry, and placed in tared tubes containing 3.0 ml of 30% (w/v)

KOH and digested at 90°C for 3 h. Two portions of maternal liver (0.7–1.0 g) were excised and treated as described above. Maternal blood was drawn by puncture of the descending aorta, and 0.5 ml was deproteinized with 4.5 ml of 95% ethanol.

Isolation of glycogen

Glycogen was isolated from the KOH digest by precipitation with 80% ethanol. The pellet was dissolved in 4.5 ml of water, neutralized with 1 M-H₂SO₄ and the glycogen reprecipitated in the presence of 65% ethanol for 12 h at 4°C. The pellet was finally dissolved in 5.0 ml of water.

Analytical procedures

Glucose in the deproteinized blood and that liberated by amyloglucosidase treatment of the purified hepatic glycogen was measured by using a glucose oxidase method (Clark *et al.*, 1974). L(+)-Lactate in blood was determined enzymically by the method of Hohorst (1963). Radioactivity in liver glycogen and in foetal and maternal blood was assayed in a Triton-based liquid-scintillation 'cocktail' (Philippidis *et al.*, 1972).

Column chromatography

Supernatant from the deproteinized blood was passed through three ion-exchange columns: Amberlite CG-120, Dowex 1 (acetate form) and Dowex 1 (borate form). Water, glucose, lactate, pyruvate and amino acids were eluted as described previously (Katz & Wals, 1972; Clark *et al.*, 1975).

Statistical methods

The statistical significance of differences between experimental groups was determined by the analysis of variance (Brownlee, 1949). A *P* value of 0.05 or less was taken as the criterion of statistical significance.

The statistical significance of differences between the rates of degradation of ¹⁴C-labelled, ³H-labelled and total glycogen were determined by performing a linear regression on the appropriate data for each separate litter. These results were converted into rates of glycogen utilization and were compared by the Kruskal–Wallis non-parametric analysis of variance (Kruskal & Wallis, 1952, 1953).

Results and discussion

The mean birth weight of the caesarian-delivered *gsd/gsd* pups that were killed immediately (4.98 ± 0.10 g; *n* = 18) was not statistically different from that of the controls (4.79 ± 0.095 g; *n* = 17). The unaffected animals remained at 4.8 g, but the weight of the *gsd/gsd* neonatal rats was decreased by 9% (*P* < 0.05) after 10 h of food deprivation. This loss was not reflected in the livers of these pups, which

remained at 310mg (Table 1). On the other hand, the weight of hepatic tissue from the control neonatal rats was decreased by 40% at 10h ($P < 0.001$; Table 1). The differences in liver weight between the *gsd/gsd* and control neonatal rats can be attributed to the glycogen content: this was unchanged in the affected pups, but was decreased by over 90% in the controls (Table 1; see also den Otter & van Boxtel, 1971; Watts & Gain, 1976).

Glycogen degradation in the neonatal liver

In these experiments liver glycogen was labelled with [6- ^3H]glucose 2.5 days before birth and with ^{14}C from [U- ^{14}C]galactose immediately after delivery (see the Materials and methods section). In the affected pups ^3H in liver glycogen was not altered during the 10h of food deprivation. Similar results were obtained when ^{14}C radioactivity was monitored in the same samples (Table 1). In this case the isotope was maintained in the neonatal hepatic glycogen, at the 20min value, throughout the investigation. These isotopic data demonstrate that the hepatic glycogen in the *gsd/gsd* neonatal rat cannot be mobilized either at birth or in the first 10h after birth. This confirms the analytical data (Table 1), which demonstrate that the polysaccharide is maintained at about 670 μmol of glycogen glucose/g of liver (12% w/w) during the first 10h *post partum*.

In the control pups the incorporation of [6- ^3H]glucose into hepatic glycogen was less than one-half that of the *gsd/gsd* neonatal rats (Table 1). Possible reasons for this difference are presented below in the Discussion section. The [6- ^3H]glucose, which had been administered to the mothers 2.5 days previously, was maintained in the liver glycogen of the control neonatal rats at the zero-time value for 5h, but was then decreased by over 80% during the second 5h of starvation (Table 1). The second isotope in this polysaccharide, ^{14}C from [U- ^{14}C]galactose, was decreased by nearly the same amount (83%), but in this case it was within the first 5h after birth (Table 1). Neither of these patterns of polysaccharide metabolism agree with the analytical determinations; these showed that the glycogen was degraded in a nearly linear manner from 9% (w/w) at birth to 0.8% (w/w) 10h later (Table 1). Non-parametric analysis of variance of the rates of glycogen utilization during the first 5h of food deprivation demonstrated that the rate of breakdown of total glycogen was significantly different from that of ^3H -labelled glycogen ($P < 0.01$) and that of ^{14}C -labelled glycogen ($P < 0.01$).

In control neonatal rats that were killed immediately after birth and placed in an incubator at 37°C there was a 29% decrease in hepatic glycogen after 1h and a 34% decrease after 10h (Table 1). The polysaccharide was also degraded in the dead *gsd/gsd* neonatal rats; in this case glycogen was

decreased by 12% at 1h and by 22% after 10h (Table 1). Although the decreases in liver glycogen in neonatal rats that had been dead for 10h were highly significant statistically ($P < 0.001$) the ^3H content of the polysaccharide was not significantly diminished (Table 1). Very similar changes are obtained when these analytical results are compared by the paired Student's *t* test. In this case hepatic glycogen was decreased by 21% ($P < 0.001$; $n = 5$) in the affected neonatal rats and by 35% ($P < 0.001$; $n = 6$) in the controls. Although this method of statistical analysis also demonstrated that there was no significant change in the ^3H -labelled glycogen in the dead control neonatal rats, it revealed that the labelled polysaccharide was decreased by 12.5% ($P < 0.05$) in the *gsd/gsd* pups 10h after death.

These results on glycogen catabolism in the neonatal rodent liver (Table 1) suggest that glycogenolysis proceeds in an ordered or sequential manner in the live and dead *GSD/GSD* neonatal rats. This pattern of metabolism is indicative of phosphorolysis (Devos & Hers, 1979). There is no evidence of glycogen degradation in liver from the live *gsd/gsd* neonatal rats (Table 1), but the random pattern of glycogenolysis *post mortem* (see above) suggests that a hydrolytic mechanism [possibly α -glucosidase (Lejeune *et al.*, 1963) or α -amylase (Rutter *et al.*, 1961)] is present in the hepatic tissue of these neonatal animals (Devos & Hers, 1980a).

Metabolism of blood glucose and lactate

In addition to comparing the metabolism of hepatic glycogen in the newborn *gsd/gsd* and *GSD/GSD* rat pups, these studies were extended to encompass the fate of glucose, lactate, ^{14}C label and ^3H label in the neonatal blood during the first 10h of life (Table 2). The concentration of ^3H label, from [6- ^3H]glucose, in blood from the *gsd/gsd* neonatal rats was over 15% higher ($P < 0.01$) than that of the control pups (Table 2). The radioisotope label remained at this concentration throughout the 10h of food deprivation in the affected neonatal rats, but in the control pups it was significantly increased by 11% ($P < 0.001$) at 10h (Table 1). This increase in ^3H label concentration in blood in the control animals coincides with the marked decrease in ^3H label in liver glycogen during the second 5h of food deprivation (Table 1). Column chromatography of the ethanolic blood supernatants from both the affected and control neonatal rats revealed that only the 10h sample from the control neonatal animals had appreciable amounts of ^3H in the glucose fraction; this accounted for 5–7% of the total blood ^3H . In the other supernatants most of the ^3H label (95–97%) was eluted in the $^3\text{H}_2\text{O}$ fraction, and only 2–3% was present in the glucose fraction.

The data in Table 2 indicate that the half-life of ^{14}C label from [U- ^{14}C]galactose in blood from the

Table 1. *Effect of food deprivation on liver weight and the metabolism of liver glycogen in neonatal gsd/gsd and control (GSD/GSD) neonatal rats*
 Hepatic glycogen, labelled with $[6\text{-}^3\text{H}]\text{glucose}$ at 19.5 days of gestation and ^{14}C from $[\text{U-}^{14}\text{C}]\text{galactose}$ after birth (Devos & Hers, 1974), was isolated and assayed as described in the Materials and methods section. Values are the means $\pm$ s.e.m. for the numbers of observations given in parentheses (means $\pm$ s.d. for two observations). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ for gsd/gsd versus control or for zero time versus other time intervals; N.S., not significant.

Time after birth (min)	Liver wt. (mg)		Liver glycogen (μmol of glucose equiv./g of liver)		$10^{-3} \times ^3\text{H}$ in liver glycogen (c.p.m./g of liver)		$10^{-3} \times ^{14}\text{C}$ in liver glycogen (c.p.m./g of liver)	
	Affected rats (gsd/gsd)	Control rats (GSD/GSD)	Affected rats (gsd/gsd)	Control rats (GSD/GSD)	Affected rats (gsd/gsd)	Control rats (GSD/GSD)	Affected rats (gsd/gsd)	Control rats (GSD/GSD)
0	297 $\pm$ 22 (7)	N.S. 271 $\pm$ 15 (7)	672 $\pm$ 11 (7)	*** 515 $\pm$ 14 (7)	120.9 $\pm$ 18.4 (7)	*** 33.2 $\pm$ 6.8 (7)		
20	321 $\pm$ 28 (6)	* 247 $\pm$ 13 (6)	675 $\pm$ 29 (6)	*** 483 $\pm$ 40 (6)	123.1 $\pm$ 24.8 (6)	* 42.3 $\pm$ 9.2 (6)	41.1 $\pm$ 7.1 (5)	N.S. 66.9 $\pm$ 14.9 (6)
60	329 $\pm$ 14 (7)	*** 246 $\pm$ 13 (7)	685 $\pm$ 21 (7)	*** 475 $\pm$ 30 (7)	114.0 $\pm$ 16.8 (7)	*** 49.1 $\pm$ 8.9 (6)	38.6 $\pm$ 4.9 (6)	* 57.4 $\pm$ 4.3 (6)
150	297 $\pm$ 8 (5)	*** 231 $\pm$ 11 (4)	645 $\pm$ 16 (5)	** 401 $\pm$ 37 (4)	90.9 $\pm$ 19.8 (5)	N.S. 52.9 $\pm$ 3.3 (4)	49.7 $\pm$ 7.5 (5)	N.S. 31.4 $\pm$ 4.6 (4)
300	318 $\pm$ 20 (2)	*** 212 $\pm$ 9 (5)	676 $\pm$ 1 (2)	*** 274 $\pm$ 42 (5)	87.4 $\pm$ 28.0 (2)	N.S. 41.6 $\pm$ 11.1 (5)	38.1 $\pm$ 0.8 (2)	*** 11.6 $\pm$ 1.1 (5)
600	309 $\pm$ 5 (7)	*** 165 $\pm$ 6 (7)	702 $\pm$ 14 (7)	*** 44 $\pm$ 24 (7)	125.9 $\pm$ 14.7 (7)	*** 6.4 $\pm$ 2.0 (7)	31.5 $\pm$ 5.7 (7)	*** 5.8 $\pm$ 1.1 (7)
60 (dead)	329 $\pm$ 15 (5)	* 262 $\pm$ 21 (4)	593 $\pm$ 29 (5)	*** 367 $\pm$ 37 (4)	77.6 $\pm$ 19.2 (5)	N.S. 41.3 $\pm$ 4.6 (4)		
600 (dead)	352 $\pm$ 20 (6)	N.S. 297 $\pm$ 22 (6)	526 $\pm$ 14 (6)	*** 340 $\pm$ 16 (6)	90.8 $\pm$ 17.1 (6)	* 41.1 $\pm$ 8.3 (6)		
Mother			579 $\pm$ 13 (5)	*** 21 $\pm$ 8 (7)	2.4 $\pm$ 0.9 (5)	* 0.3 $\pm$ 0.05 (6)		

Table 2. ^{14}C label, ^3H label, glucose and lactate in blood from neonatal *gsd/gsd* and control (*GSD/GSD*) rat pups during 10 h of food deprivation. Foetuses were delivered at term by caesarian section of ether-anaesthetized *gsd/gsd* or control (*GSD/GSD*) does and placed in an incubator at 37°C . At the appropriate time intervals the neonatal rats were weighed and decapitated, and blood was collected at the neck. Measurements were made as described in the Materials and methods section. Values are the means $\pm$ s.e.m. for the numbers of observations given in parentheses (means $\pm$ s.d. for two observations). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ for *gsd/gsd* versus *GSD/GSD* or for zero time versus other time intervals; N.S., not significant.

Time after birth (min)	$10^{-3} \times ^3\text{H}$ in blood (c.p.m./ml)		$10^{-3} \times ^{14}\text{C}$ in blood (c.p.m./ml)		Blood glucose ($\mu\text{mol/ml}$)		Blood lactate ($\mu\text{mol/ml}$)	
	Affected rats (<i>gsd/gsd</i>)	Control rats (<i>GSD/GSD</i>)	Affected rats (<i>gsd/gsd</i>)	Control rats (<i>GSD/GSD</i>)	Affected rats (<i>gsd/gsd</i>)	Control rats (<i>GSD/GSD</i>)	Affected rats (<i>gsd/gsd</i>)	Control rats (<i>GSD/GSD</i>)
0	64.7 ± 3.1 (16)	** 55.3 ± 1.0 (16)			2.01 ± 0.10 (16)	*** 3.75 ± 0.17 (17)	6.42 ± 0.82 (5)	N.S. 7.56 ± 0.56 (7)
20	63.5 ± 4.5 (6)	N.S. 55.9 ± 1.0 (6)	73.2 ± 6.5 (6)	N.S. 89.9 ± 13.6 (6)	1.50 ± 0.12 (6)	*** 5.31 ± 0.44 (6)	3.87 ± 0.78 (2)	* 6.77 ± 0.10 (3)
	N.S.	N.S.			*	***	N.S.	N.S.
60	65.9 ± 4.8 (7)	* 54.8 ± 0.9 (7)	25.8 ± 4.6 (7)	* 38.3 ± 1.9 (7)	0.41 ± 0.19 (6)	*** 2.34 ± 0.17 (7)	3.20 ± 1.04 (3)	N.S. 2.70 ± 0.41 (4)
	N.S.	N.S.	***	***	***	***	*	***
150	67.8 ± 8.6 (5)	N.S. 56.9 ± 2.0 (4)	8.7 ± 2.3 (5)	* 19.5 ± 3.6 (4)	0.29 ± 0.08 (5)	*** 2.68 ± 0.36 (4)		1.45 ± 0.53 (2)
	N.S.	N.S.	***	***	***	*		***
300	63.7 ± 20.3 (2)	N.S. 57.1 ± 0.3 (5)	5.9 ± 1.1 (2)	N.S. 12.1 ± 1.9 (5)	0.83 ± 0.03 (2)	* 3.09 ± 0.41 (5)		
	N.S.	N.S.	***	***	***	N.S.		
600	66.9 ± 6.0 (7)	N.S. 61.5 ± 1.7 (7)	4.6 ± 0.6 (7)	N.S. 6.2 ± 0.9 (7)	0.77 ± 0.20 (7)	*** 1.73 ± 0.17 (7)	1.68 ± 1.02 (3)	N.S. 1.39 ± 0.79 (4)
	N.S.	***	***	***	***	***		***
Mother	61.4 ± 5.3 (4)	N.S. 55.0 ± 0.5 (6)			4.95 ± 0.42 (5)	N.S. 5.48 ± 0.57 (7)	1.14 ± 0.39 (3)	N.S. 1.49 ± 0.13 (4)
	N.S.	N.S.			***	***	***	***

gsd/gsd neonatal rats is less than that in blood from the *GSD/GSD* pups. At the 60 min and 150 min samplings the concentration of ^{14}C label in blood from the controls was significantly higher ($P < 0.05$) than that from the affected pups (Table 2). This probably results from the release of $[\text{U-}^{14}\text{C}]\text{glucose}$ from liver glycogen, as glycogenolysis occurs only in the control neonatal rats (Table 1). The half-life of $[\text{U-}^{14}\text{C}]\text{glucose}$ in rat blood is about 4 times that of $[\text{U-}^{14}\text{C}]\text{galactose}$ (Devos & Hers, 1979), and this would tend to elevate the concentrations of ^{14}C label in the blood from the *GSD/GSD* pups at 60 and 150 min. In the final blood sample (10 h) concentrations of ^{14}C label were nearly identical for both strains of rats (Table 2).

Previous work has demonstrated that the blood glucose concentrations in *gsd/gsd* rats fed *ad libitum* can be significantly lower than those of control (*GSD/GSD*) rats, but this difference is not increased during extended periods of food deprivation (Clark *et al.*, 1980; Malthus *et al.*, 1980). In the control neonatal rats, blood glucose concentrations at birth were significantly lower ($P < 0.001$) than those of their mothers, but they rose to the maternal value within 20 min of delivery (Table 2). In the subsequent samples (60 and 150 min) the concentration of the hexose was decreased by about 50%, and after 10 h of food deprivation it was further diminished to less than 2 mM (Table 2). The *gsd/gsd* neonatal rats were hypoglycaemic throughout the course of the investigation (Table 2). At delivery the concentration of glucose in their blood was about one-half that of the controls, and it was decreased by 80% within 60 min. A nadir of less than 0.3 mM was observed at 150 min, but the glucose concentrations were increased more than 2.5-fold during the final 5 h of food deprivation (Table 2).

Gain *et al.* (1980) have shown that the concentrations of glucose in blood from the *gsd/gsd* rat foetuses at days 17, 19 and 22 of gestation (2.7, 1.7 and 1.9 mM respectively) were 30–65% ($P < 0.005$) lower than those from the control pups. It was further demonstrated that the concentration of the hexose in the maternal *gsd/gsd* rats was also decreased by 35–50% ($P < 0.005$) over the same period of gestation. At day 19 the concentration of glucose in the *gsd/gsd* does was 3.98 ± 0.09 mM ($n = 10$), whereas that in the controls was 5.99 ± 0.22 mM ($n = 8$) (Gain *et al.*, 1980). If this were also true in the present investigation, then the specific radioactivity of $[\text{6-}^3\text{H}]\text{glucose}$ in the foetal *gsd/gsd* rats would be 50% greater than that from the controls. This would explain, at least in part, why the incorporation of $[\text{6-}^3\text{H}]\text{glucose}$ into the hepatic glycogen of the affected neonatal rats at day 19.5 was more than double that found in the controls (Table 1).

Another mechanism that could account for this would be the presence of more glycogen synthase *a* in the liver of the affected foetus at day 19 of gestation. Although total glycogen synthase activity (expressed as units/mg of protein) is similar in both *gsd/gsd* and control foetuses at this period of development, the active form of this enzyme is actually diminished in hepatic tissue from the affected foetuses (K. R. Gain & C. Watts, personal communication). It is apparent from these results that differences in hepatic glycogen synthase *a* are not responsible for the increased incorporation of $[\text{6-}^3\text{H}]\text{glucose}$ (Table 1). Another possible explanation is that the labelled glucose that was incorporated into the liver glycogen of the control foetuses might be released by phosphorolysis and further catabolized (Watts & Gain, 1976). As this polysaccharide is not degraded in either the neonatal (Table 1) or the mature *gsd/gsd* rat (Clark *et al.*, 1980; Malthus *et al.*, 1980), it is unlikely that this would occur in the affected foetus during the latter stages of prenatal development.

The changes in blood lactate concentrations during the 10 h of starvation were similar in both the *gsd/gsd* and the *GSD/GSD* neonatal rats (Table 2). The lactate concentrations were greatly elevated after delivery, but were decreased by more than 50% within 1 h. These results demonstrate that this compound is rapidly metabolized in these pups after birth, and this metabolism presumably has a sparing effect on blood glucose. This is not apparent in the affected neonatal rats, where the concentration of the hexose fell by 80% within 60 min of birth (Table 2), but in the control pups there was no significant removal of glucose from the hepatic glycogen stores during this time (Table 1).

Perinatal synthesis and degradation of hepatic glycogen

There has been a resurgence of interest over the last 2 years on whether glycogen reserves are synthesized and degraded in an ordered or random manner (Devos & Hers, 1979, 1980a,b) and on the role of lysosomal α -glucosidase in the mobilization of this polysaccharide in the newborn rat (Hers & de Barsey, 1973; Devos & Hers, 1980a; Iwamasa *et al.*, 1980). The results of the present investigation add further information to both these areas of research. The isotopic and analytical data obtained with glycogen from the liver of the control neonatal rats (Table 1) show that this polysaccharide, like that from adult mice and rats (Devos & Hers, 1979), was synthesized and degraded in an ordered (sequential) manner. Thus $[\text{6-}^3\text{H}]\text{glucose}$, which was the first isotopic label incorporated into the foetal *GSD/GSD* glycogen, was the last out, whereas ^{14}C label from $[\text{U-}^{14}\text{C}]\text{galactose}$, the last in, was nearly completely removed within 5 h of birth (Table 2).

These results contrast with those from a similar study by Devos & Hers (1980a); they found that the glycogen from their neonatal rat pups was degraded in a random, presumably lysosomal, manner. The only differences between the present study and that of Devos & Hers (1980a) were that the latter workers labelled foetal glycogen with [^{14}C]glucose and they delivered their fetuses at day 21.5 of gestation from decapitated mothers (cf. the Materials and methods section).

There is no evidence for either phosphorolytic or hydrolytic degradation of glycogen in hepatic tissue from live *gsd/gsd* neonatal rats (Table 1), but the results obtained with affected neonatal rats that had been dead for either 1 or 10h (Table 1) demonstrate that liver from the affected animals does contain some enzymic mechanism for the degradation of hepatic glycogen. The nature of this mechanism remains to be elucidated.

We are indebted to Professor H. G. Hers and Dr. R. M. Smith for helpful comments and criticisms of this work, and to Dr. P. Baghurst for his help with the statistical analyses.

References

- Bashan, N., Gross, Y., Moses, S. & Gutman, A. (1979) *Biochim. Biophys. Acta* **587**, 145–154
- Brownlee, K. A. (1949) *Industrial Experimentation*, 4th edn., p. 74, H.M. Stationery Office, London
- Clark, D. G., Rognstad, R. & Katz, J. (1974) *J. Biol. Chem.* **249**, 2028–2036
- Clark, D. G., Lee, D., Rognstad, R. & Katz, J. (1975) *Biochem. Biophys. Res. Commun.* **67**, 212–219
- Clark, D. G., Topping, D. L., Illman, R. J., Trimble, R. P. & Malthus, R. S. (1980) *Metab. Clin. Exp.* **29**, 415–420
- Dawkins, M. J. R. (1963) *Ann. N.Y. Acad. Sci.* **111**, 203–211
- den Otter, W. & van Bostel, A. B. Th. G. (1971) *Experientia* **27**, 1271–1272
- Devos, P. & Hers, H. G. (1974) *Biochem. J.* **140**, 331–340
- Devos, P. & Hers, H. G. (1979) *Eur. J. Biochem.* **99**, 161–167
- Devos, P. & Hers, H. G. (1980a) *Biochem. J.* **192**, 177–181
- Devos, P. & Hers, H. G. (1980b) *Biochem. Biophys. Res. Commun.* **95**, 1031–1036
- Gain, K. R., Malthus, R. & Watts, C. (1980) *Proc. Univ. Otago Med. Sch.* **58**, 39–41
- Gilbert, M. & Bourbon, J. (1978) *Biochem. J.* **176**, 785–789
- Goldwater, W. H. & Stetten, D., Jr. (1947) *J. Biol. Chem.* **169**, 723–738
- Goodall, C. M., Christie, G. S. & Hurley, J. V. (1975) *J. Pathol.* **116**, 239–251
- Hers, H. G. & de Barsy, Th. (1973) in *Lysosomal and Storage Diseases* (Hers, H. G. & Van Hoof, V. F., eds.), pp. 141–171, Academic Press, London and New York
- Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 266–270, Academic Press, New York and London
- Iwamasa, T., Tsuru, T., Hamada, T. & Takeuchi, T. (1980) *Pathol. Res. Pract.* **167**, 363–373
- Jezequel, A., Arakawa, K. & Steiner, J. W. (1965) *Lab. Invest.* **14**, 1894–1930
- Katz, J. & Wals, P. A. (1972) *Biochem. J.* **128**, 879–899
- Kruskal, W. H. & Wallis, W. A. (1952) *J. Am. Stat. Assoc.* **47**, 583–621
- Kruskal, W. H. & Wallis, W. A. (1953) *J. Am. Stat. Assoc.* **48**, 907–911
- Lejeune, N., Thinès-Sempoux, D. & Hers, H. G. (1963) *Biochem. J.* **86**, 16–21
- Malthus, R. & Clark, D. G. (1977) *Proc. Univ. Otago Med. Sch.* **55**, 31–32
- Malthus, R., Clark, D. G., Watts, C. & Sneyd, J. G. T. (1980) *Biochem. J.* **188**, 99–106
- Philippidis, H., Hanson, R. W., Reshef, L., Hopgood, M. F. & Ballard, F. J. (1972) *Biochem. J.* **126**, 1127–1134
- Phillips, M. J., Unaker, N. Y., Doornewaard, G. & Steiner, J. W. (1967) *J. Ultrastruct. Res.* **19**, 142–165
- Rutter, W. J., Arnold, M., Brosemer, R. W. & Miller, J. A. (1961) *J. Biol. Chem.* **236**, 1259–1263
- Shelley, H. J. (1961) *Br. Med. Bull.* **17**, 137–143
- Snell, K. & Walker, D. G. (1973) *Biochem. J.* **132**, 739–752
- Watts, C. & Gain, K. R. (1976) *Biochem. J.* **160**, 263–270