Glycogen Metabolism in the Liver of the Developing Rat

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1. The total activity of glycogen synthase increased 20-fold from day 17 of gestation to birth at day 22, with a further increase of 18% in the 24 h after birth. Active synthase (*I*) rose 45-fold to a maximum at day 21, fell 40% before birth, and then increased by a similar amount 24 h after birth. The fraction of synthase in the active form correlated very well with the deposition of glycogen in the liver. 2. Total phosphorylase had a similar developmental pattern to total synthase with an 18-fold increase from day 17 to day 22. The appearance of active phosphorylase showed a lag-period compared with total phosphorylase and did not increase significantly until day 20. The fraction of phosphorylase in the active form did not correlate at all with glycogen deposition or mobilization. 3. There was a close relationship between the ratio of phosphorylase *a*/synthase *I* and the glycogen content of the liver. An increase or decrease in this ratio would result in glycogenolysis or glycogenesis respectively. 4. It is postulated that a cycle between the two enzymes under basal conditions could exist which permits a continuous turnover of glycogen. Such a system would explain why active phosphorylase is always seen, even under conditions of net glycogen synthesis. The differences in hormone sensitivity of synthase and phosphorylase would also be accounted for as only one enzyme would have to respond acutely to hormonal influences.

Glycogen stores in the liver of the foetus increase during the later stages of gestation in many species, including the rat (Shelley, 1961), and are mobilized rapidly at birth to supply glucose to the newborn in the hours immediately after birth (Dawkins, 1963; Ballard & Oliver, 1963; Devos & Hers, 1974). The large and clearly defined changes in glycogen metabolism over a known time-span offer a good model for the study of enzymological aspects of glycogen turnover. Many of the previous reports on glycogen metabolism in foetal rat liver, however, have been incomplete studies on the two major enzymes of glycogen metabolism, glycogen synthase and phosphorylase. Either the changes in only one of the enzymes, or in only the active or inactive forms of both enzymes, were measured (Dawkins, 1963; Ballard & Oliver, 1963; Novak *et al.*, 1972). There have been only two investigations on both active and inactive forms of both enzymes in the liver of foetal rats (Schwartz & Rall, 1973; Devos & Hers, 1974). There was a limited agreement in the developmental patterns of these enzymes, but there were also major quantitative differences, many of which appeared to arise from methodological differences. Neither of the reports established or claimed any interrelationship between the synthase and phosphorylase in the overall control of glycogen deposition or mobilization.

In the present work, a study has been made of the total and active synthase and phosphorylase enzymes

in relation to the glycogen content of the liver in foetal rats over the last 6 days of gestation and the first 24 h after birth. The results suggest that the ratio of the active forms of phosphorylase and synthase may be of major importance in regulating glycogen storage in the liver and that, under basal conditions, there could be a continual cycling of glucose through glycogen.

Experimental

Materials

Animals. All studies were made on rats of a derived Wistar strain. Pregnancy was dated from the observation of a copulation plug in the vagina after the females were caged with a male overnight, that day being taken as day 0 of gestation. Birth of a litter normally occurred at some time on day 22 of gestation. Tissue from non-pregnant adult females was used for all control data for adults.

Chemicals. UDP-[U-¹⁴C]glucose was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Bovine serum albumin (fraction V), UDP-glucose, glucose 1-phosphate, glucose 6-phosphate and shellfish glycogen were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Glycogen solutions were treated with Amberlite Monobed Resin (BDH Chemicals, Poole, Dorset, U.K.) before use to remove sugar phosphate impurities.

Methods

Preparation of tissue homogenates. Livers were taken from foetuses after anaesthetizing the mother with pentobarbital (Nembutal; Abbott Laboratories, New Zealand) given intraperitoneally at 10mg/100g body wt. The livers were removed from a midline incision of the abdomen by a pair of fine scissors, care being taken not to include any non-hepatic tissue. Naturally born rats were left with the mother until the time of the experiment, when their livers were removed under ether anaesthesia. Whenever possible, tissues were taken between 11.00 and 13.00h to minimize any influence of diurnal variation on the results.

Livers from foetal and newborn rats were immediately placed in liquid N₂ if they were to be used for phosphorylase or glycogen assays. Frozen livers (4–6) were pooled and homogenized in 3 vol. of ice-cold 50mM-Tris/HCl, pH 8.2, containing 5mM-EDTA, 50mM-NaF and 15mM-cysteine for phosphorylase studies. For glycogen synthase measurements, the livers were washed immediately on removal by swirling rapidly in the ice-cold buffer and touch-drying on Whatman no. 52 filter paper before freezing to remove the glycogenolytic contaminant described elsewhere (Gain, 1976). These frozen livers were pooled and homogenized in 3 vol. of ice-cold 50mM-Tris/HCl, pH 7.8, containing 5mM-EDTA and 100mM-NaF. Samples of liver from adult rats were removed under Nembutal anaesthesia and frozen immediately in liquid N₂. They were then homogenized in the appropriate buffer.

All homogenates were centrifuged at 3000g for 10min at 0°C; the supernatants were decanted and kept at 0°C until assayed within 2h.

Enzyme assays. (a) Glycogen synthase (EC 2.4.1.11) was measured by incorporation of [U-¹⁴C]glucose from UDP-[U-¹⁴C]glucose into glycogen. The labelled glycogen was measured after adsorption on filter paper (Thomas *et al.*, 1968).

(b) Active synthase (I) was assayed at pH 7.8 by adding 100μl of homogenate supernatant to 200μl of reaction mixture containing 0.9μmol of UDP-glucose (containing 0.2μCi of UDP-[U-¹⁴C]glucose/μmol), 10μmol of Tris, 1μmol of EDTA, 3μmol of Na₂SO₄ and 2mg of shellfish glycogen; pH was adjusted with HCl. Incubation time was 10min at 30°C.

(c) Total synthase (I + D) was assayed at pH 7.8 by adding 100μl of a further 1:5 (v/v) dilution of the homogenate supernatant to 200μl of reaction mixture containing 1μmol of UDP-glucose (containing 0.1μCi of UDP-[U-¹⁴C]glucose/μmol of UDP-glucose), 10μmol of Tris, 1μmol of EDTA, 1.5μmol of glucose 6-phosphate and 2mg of glycogen; pH was adjusted with HCl. Incubation time was 4min at 30°C.

Synthase activities are expressed as munits/mg of protein or units/whole liver. A unit of enzyme activity catalysed the incorporation of 1μmol of glucose from UDP-glucose into glycogen in 1min under the conditions of the assay.

(d) Phosphorylase (EC 2.4.1.1) was assayed by the measurement of the P_i released during incorporation of glucose 1-phosphate into glycogen (Buell *et al.*, 1958).

Table 1. *Effects of NaF and caffeine on the assay of phosphorylase a and b*

Liver homogenates were prepared from 21-day foetuses and adult liver in the absence of NaF. One half of each homogenate was diluted 1:4 (v/v) with NaF-containing buffer and incubated with phosphorylase b kinase to produce mainly phosphorylase a. The other half was incubated at 37°C for 20min to produce mainly phosphorylase b, and this was diluted 1:4 with NaF-containing buffer before assay for phosphorylase activity at pH 6.1. Caffeine was added to a final concentration of 0.5mM. Phosphorylase activity is expressed as munits/mg of protein.

			Phosphorylase activity					
	Homogenate	Final NaF (mM)	...	6.7	20	40	70	140
Foetal	Phosphorylase <i>a</i>							
	–Caffeine			175	169	189	167	164
	+Caffeine			177	167	172	174	140
	Phosphorylase <i>b</i>							
	–Caffeine			22	23	28	25	17
	+Caffeine			24	24	27	27	18
Adult	Phosphorylase <i>a</i>							
	–Caffeine			257	233	253	250	236
	+Caffeine			259	261	276	236	223
	Phosphorylase <i>b</i>							
	–Caffeine			52	49	48	40	40
	+Caffeine			49	44	46	37	34

(e) Active phosphorylase (*a*) was assayed at pH 6.1 in the homogenate supernatant after a further 1:35 (v/v) dilution in 200 mM- β -glycerophosphate, 5 mM-EDTA, 20 mM-NaF, 400 mM-sucrose, 15 mM-cysteine, pH 6.1. A portion (100 μ l) of the diluted supernatant was added to 200 μ l of reaction mixture containing 20 μ mol of glucose 1-phosphate, 40 μ mol of NaF and 2 mg of glycogen. Incubation time was for 30 min at 37°C.

(f) Total phosphorylase (*a*+*b*). The procedure involved incubation of the undiluted homogenate supernatant with a partially purified phosphorylase *b* kinase at final substrate concentrations of 12 mM-ATP and 10 mM-MgSO₄ for 15 min at 37°C. The incubation mixture was then diluted 1:21 (v/v) in the ice-cold buffer used in the phosphorylase *a* assay and assayed for phosphorylase activity as described above.

Stalman & Hers (1975), using Sephadex G-25 filtrates of homogenates of adult rat liver, showed that phosphorylase *b* had 25% of the activity of phosphorylase *a* when assayed at pH 6.1 in the presence of 150 mM-NaF and this was doubled when 1 mM-5'-AMP was also present. The stimulatory effects were virtually eliminated by 0.5 mM-caffeine. In our assay system the final NaF concentration was 130 mM, and if it was assumed that all the ATP added in the phosphorylase *b* kinase step was degraded, the final 5'-AMP concentration in the phosphorylase incubation mixture might have been in excess of 0.5 mM. Under these conditions it is conceivable that phosphorylase *b* could contribute significantly to the measured phosphorylase *a* activity.

To test this, two homogenates of liver from foetal and adult rats with mainly phosphorylase *a* in one and phosphorylase *b* in the other were assayed for phosphorylase activity over a range of NaF concentrations in the presence and absence of caffeine as described in Table 1. Phosphorylase *b* in homogenates from both adult and foetal liver was not stimulated at NaF concentrations up to those used in the final phos-

phorylase assay. Further, 0.5 mM-caffeine produced no significant decrease in phosphorylase *a* or *b* activities, indicating the absence of any 5'-AMP stimulation. From these results it was concluded that there was no major contribution of phosphorylase *b* to the measurement of phosphorylase *a* in our system.

Phosphorylase activities are expressed as munits/mg of protein or units/whole liver. A unit of activity catalysed the release of 1 μ mol of P_i from glucose 1-phosphate in 1 min under the conditions of the assay.

The phosphorylase *b* kinase was prepared from rat skeletal muscle by a modification of the procedure described for rabbit skeletal muscle by Krebs *et al.* (1964). The modification consisted of carrying out the first acid precipitation step at pH 5.5 instead of pH 6.1, which greatly increased the yield of enzyme from the rat muscle (Gain, 1976).

Assay of liver glycogen. Frozen liver was weighed rapidly and the glycogen content measured by the procedure of Lo *et al.* (1970) for muscle biopsy samples.

Assay of liver protein. The soluble protein content of liver homogenates was measured by the method of Lowry *et al.* (1951) with bovine serum albumin fraction V as standard.

The statistical significance of all data was determined by Student's *t* test for unpaired data, a *P* value of <0.05 being taken as significant.

Results

The liver of the foetal rat increases in weight almost fivefold over the last 6 days of gestation (Table 2). This compares with a sevenfold increase in the total weight of the foetus over the same period. There was a 20% loss in weight in the 24 h after birth and this was due, presumably, to the loss of glycogen. Table 2 also shows the deposition of glycogen in the liver.

Table 2. Liver weight, soluble protein and glycogen content in foetal and newborn rats

Values are the mean $\pm$ S.E.M. of observations on the number of litters shown for each age. In any one litter, some livers were frozen immediately in liquid N₂ and pooled for the glycogen assay, and other livers were weighed and then pooled for protein assay. Samples on day 22 were all taken before birth, and those at +12 h and +24 h postnatal were taken at various times in the first 12 h and 12–24 h periods after birth.

	No. of litters	Day of gestation						Postnatal		Adult
		17	18	19	20	21	22	+12h	+24h	
Liver wet wt. (mg)	...	12	10	16	9	10	10	8	14	10
Glycogen (mg/g of liver)		55 $\pm$ 2	106 $\pm$ 2	132 $\pm$ 5	215 $\pm$ 20	286 $\pm$ 18	259 $\pm$ 9	—	202 $\pm$ 28	—
Protein (mg/g wet wt.)		1.7 $\pm$ 0.1	5.2 $\pm$ 0.7*	21 $\pm$ 2*	52 $\pm$ 8*	69 $\pm$ 6†	68 $\pm$ 5	22 $\pm$ 3§	15 $\pm$ 2§	35 $\pm$ 9
		132 $\pm$ 7	104 $\pm$ 6†	117 $\pm$ 4†	112 $\pm$ 4	120 $\pm$ 6	108 $\pm$ 10	190 $\pm$ 8*	181 $\pm$ 11	186 $\pm$ 5

* Significantly different from previous day, *P* < 0.001.

† Significantly different from previous day, *P* < 0.005.

‡ Significantly different from previous day, *P* < 0.02.

§ Significantly different from adult values, *P* < 0.02.

Table 3. Liver glycogen synthase and phosphorylase activities in foetal and newborn rats

Enzyme activities, expressed as munits/mg of protein, are the mean $\pm$ S.E.M. for the number of litters studied at each age. In any one litter, some livers were frozen immediately in liquid N₂ and pooled for phosphorylase assays, and other livers were washed before freezing and pooled for synthase assays. Livers from adult rats were treated in a similar way.

	Day of gestation						Postnatal	Adult
	17	18	19	20	21	22	+24h	
Glycogen synthase								
No. of litters ...	6	6	6	6	6	7	7	10
Total	8.9 $\pm$ 0.5	13.2 $\pm$ 1.3†	23.8 $\pm$ 0.8*	30.1 $\pm$ 1.1*	33.1 $\pm$ 2.5	39.0 $\pm$ 1.4‡	35.1 $\pm$ 1.7§	12.3 $\pm$ 0.5
Active (I)	0.26 $\pm$ 0.02	0.65 $\pm$ 0.10*	1.98 $\pm$ 0.11*	2.63 $\pm$ 0.32‡	2.35 $\pm$ 0.24	1.81 $\pm$ 0.12‡	1.98 $\pm$ 0.12§	0.95 $\pm$ 0.07
Phosphorylase								
No. of litters ...	10	8	12	5	7	5	9	10
Total	63 $\pm$ 5	101 $\pm$ 7*	132 $\pm$ 8†	213 $\pm$ 21*	204 $\pm$ 13	324 $\pm$ 52†	212 $\pm$ 20¶	268 $\pm$ 13
Active (a)	21 $\pm$ 3	40 $\pm$ 7†	30 $\pm$ 5	58 $\pm$ 12†	71 $\pm$ 7	97 $\pm$ 37	110 $\pm$ 13¶	149 $\pm$ 14

* Significantly different from previous day, $P < 0.001$.

† Significantly different from previous day, $P < 0.01$.

‡ Significantly different from previous day, $P < 0.05$.

§ Significantly different from adult values, $P < 0.001$.

¶ Significantly different from adult values, $P < 0.05$.

There was very little glycogen at days 17 and 18 compared with values seen in the adult rats fed *ad libitum*, but rapid accumulation occurred over the next 3 days to amounts significantly higher than those of the adults. There was a rapid loss of glycogen at birth and during the immediate postnatal period to give low values by 24 h after birth. These results are similar to those of other workers (Shelley, 1961; Greengard & Dewey, 1970; Devos & Hers, 1974). The protein content of the liver showed a significant fall from day 17 to day 18 which is not easy to explain. The most significant finding was the very large increase in protein content of the foetal liver which occurred soon after birth and approached values seen in adult liver. This corresponded in time, and was of the same order as the fall in hepatic glycogen.

The changes in activity (expressed per mg of protein) of the total and active form of glycogen synthase and phosphorylase over late gestation and the early postnatal period are shown in Table 3. There was a steep rise in the total synthase activity from day 17 onwards with a peak of activity being seen before birth on day 22. At that age, values were three times those seen in adult liver. There was a small fall in total synthase activity in the 24 h period after birth. Activities seen in the liver of newborn rats were significantly higher than those seen in adult liver. Synthase I activity also increased from day 17 onwards but reached a peak at day 20 and then fell to significantly lower values by day 22. In contrast with the total activity, there was a rise after birth to values that were significantly higher than those seen in adult liver.

There was a 3-fold increase in total phosphorylase activity from day 17 to day 20 and a further significant rise before birth on day 22, although there was a wide variation in values seen on that day. There followed a

significant fall in total activity after birth. In contrast with the synthase, the activities seen in the foetal liver (except for day 22) were always very much less than those seen in adult liver. This was also true for phosphorylase *a*. The active enzyme showed a significant rise in activity from day 17 to 18 followed by a fall at day 19. There was then a steady rise through to birth, but again, activities seen on day 22 showed wide variation. There was no evidence of a fall in activity of phosphorylase *a* after birth, although values were significantly less than those seen in liver from adult rats.

Although the results in Table 3 indicate a large change in the activities of the total activity and active form of both enzymes when expressed in terms of protein, their interpretation can be complicated by three factors, (a) the varying protein content of the liver during gestation, particularly around the time of birth, (b) the changing contribution of glycogen to the wet weight of the liver and (c) the changing cellular composition of the organ causing an alteration in the ratio of parenchymal to haemopoietic cells (Greengard *et al.*, 1972), presuming that the enzymes reside primarily in the parenchymal cells. For these reasons it is desirable to show changes in the absolute amounts of each enzyme, as in this way changes in enzyme activity can be seen independently of other changes in the liver. The simplest way to do this is to calculate the total number of units present in the whole liver. This has been done by using the mean values at each age for the two enzymes (see Table 3) and the mean values for protein content and wet weight of the liver given in Table 2 (Figs. 1 and 2).

Total synthase showed a 20-fold increase from day 17 to birth at day 22. This compares with only a five-fold rise in liver weight over the same period and must

Fig. 1. Synthase activity of foetal liver during gestation expressed as total units/whole liver

The enzyme units/whole liver at each age were calculated from the mean activities as munits/mg of protein $\times$ mean protein content (mg/g wet wt. of liver) $\times$ mean liver wet wt. (in mg). ○, Total synthase; Δ, synthase *I*.

Fig. 2. Phosphorylase activity of foetal liver during gestation expressed as total units/whole liver

The enzyme units/whole liver at each age were calculated from the mean phosphorylase activities by the same calculation as in Fig. 1. ○, Total phosphorylase; Δ, phosphorylase *a*.

therefore represent an increase in the enzyme activity per cell in the liver. There was a further 18% rise in activity over the 24h after birth. Synthase *I* activity rose to a maximum by day 21 then fell approx. 40% at birth, but then increased an almost equivalent amount over the 24h period after birth. The maximum

Fig. 3. Active forms of synthase (a) and phosphorylase (b) as a percentage of the total activity of each enzyme

The percentage of enzyme in the active form was calculated for each homogenate from the total and active activities in munits/mg of protein. Results are shown as the mean $\pm$ S.E.M. of the number of samples in parentheses.

activity at day 21 was approx. 45 times that at day 17 and must again represent a true increase in enzyme content per cell. There appeared to be a slight lag in the appearance of the active enzyme compared with the inactive form from day 17 to day 18, but the two forms then developed in a parallel manner up to day 20.

Fig. 2 shows that the total phosphorylase has a developmental pattern similar to that of total synthase, and again, with an 18-fold increase from day 17 to day 22, there was a real increase in enzyme content per cell in the liver during gestation. There was a fall in total activity after birth, however, of approx. 10% compared with an 18% rise for total synthase. The appearance of phosphorylase *a* was slower than that of synthase *I* and also showed a definite lag-period compared with the total phosphorylase, large increases in activity not being seen until after day 19 of gestation. There was a further increase in phosphorylase *a* by 24h after birth.

Synthase *I* and phosphorylase *a* are believed to be the physiologically active forms of the respective enzymes in the liver. If there is to be a 'switch-on' mechanism operating for these enzymes in the control of glycogen metabolism, the changes in the fraction of the enzymes in their active forms should be detected during gestation and these should correlate with the glycogen deposition and mobilization. Fig. 3 shows a plot of the percentage activity in the active form for both enzymes over the period under study. For synthase, the fraction in the *I* form increases almost threefold from $3.0 \pm 0.3\%$ at day 17 to $8.7 \pm 0.9\%$ at day 20, which correlates well with the time of maximum glycogen deposition. The highest value observed was

Fig. 4. Ratio of active phosphorylase to active synthase and glycogen content of foetal liver

The phosphorylase *a*/synthase *I* ratio (●) was calculated from the mean activities of the two enzymes at each age. The mean glycogen content of the foetal liver (○) at each age has been plotted for comparison.

comparable with the fraction of active synthase ($7.7 \pm 0.5\%$) seen in livers of adult rats. There was a significant fall in the percentage of active enzyme from day 20 to term at day 22 ($P < 0.005$) with a value of $4.7 \pm 0.04\%$, which was significantly lower than adult values ($P < 0.02$). There was a small rise to $5.8 \pm 0.6\%$ in the 24 h after birth.

The good correlation that exists between the fraction of synthase in the active form and glycogen deposition was not seen with phosphorylase *a* and glycogen mobilization. At days 17 and 18 the mean fraction of phosphorylase in the active form was between 30–40%, but there was a significant fall at day 19 to $22 \pm 3\%$ ($P < 0.02$). There was then a rise to values around 30% at days 21 and 22 and no further rise was detected during the first 12 h after birth. In fact a significant rise could not be detected until 24 h after birth, with values of $53 \pm 7\%$ ($P < 0.01$). Detailed studies on the mobilization of glycogen at the time around birth in relation to the foetal blood glucose have shown that the glycogen content of the liver falls rapidly at or even just before birth (Gain & Watts, 1976) and Table 2 shows that 60% of the glycogen has gone by 12 h after birth. A significant rise in the fraction of phosphorylase in the active form did not occur until much later. These results are difficult to reconcile with a physiological role for phosphorylase *a* in the control of glycogenolysis.

There are significant activities of synthase and phosphorylase in their physiologically active forms throughout the last 6 days of gestation and after birth. It is possible that the interrelationship between the two active enzymes could be important in controlling the glycogen content of the liver. This rela-

tionship can be expressed as the ratio of phosphorylase *a*/synthase *I* activities at each age. This ratio has been compared with the measured glycogen in Fig. 4. A high enzyme ratio at days 17 and 18 was associated with very low glycogen concentrations in the liver. Over days 19–21, when the ratio was considerably decreased (2.5–4 times), active glycogen deposition occurred. Cessation of this deposition coincided with a doubling of the enzyme ratio to over 50 (days 21–22). From these results, there appears to be a direct relationship between the glycogen content of the liver and the ratio of phosphorylase *a*/synthase *I*. This is a noteworthy result in the light of a lack of correlation between the phosphorylase *a* activity and glycogen deposition and mobilization.

Discussion

The deposition of glycogen in the foetal liver and subsequent loss around birth seen in the present study is similar in pattern and degree to that reported by Snell & Walker (1973) and Devos & Hers (1974). The peak glycogen content occurred at day 21 but the exact time at which rapid glycogenolysis began in relation to the onset of birth cannot be deduced from these results, although a more detailed study over the period of birth indicated that it occurred just before or within minutes of birth (Gain & Watts, 1976).

The weight of a rat liver varies with the amount of glycogen stored in it. In the liver of a rat on a high-carbohydrate diet, glycogen accounts for 10% of the wet weight and 46% of the dry weight, and after 24 h starvation, leading to loss of all the glycogen, the liver wet weight falls by half and produces a doubling of the protein content in terms of wet weight (den Otter & van Bostel, 1971). From this it would appear that the large rise in protein in the foetal liver immediately after birth seen in the present study was a direct result of the rapid change in liver glycogen.

The changing constituents of the developing liver cause complications in the expression and interpretation of enzyme activities from one age of gestation to the next. It is not clear to what extent changes in cell type, glycogen content and protein content influence the enzyme results. In an attempt to minimize these complicating factors, the enzyme activities were expressed as units/whole liver. From these data it became clear that the very large increase in total synthase and phosphorylase activities over late gestation was a real increase in enzyme content of the liver cells. None of the previous reports have taken account of such factors. Schwartz & Rall (1973) expressed their results as units/mg of protein. On that basis, the total synthase activities were less than half those reported here and they showed no substantial increase in activity from day 19 onwards. This was probably due to interference by amylase in the radioassay of

synthase which occurs in homogenates of unwashed foetal liver (Gain & Watts, 1976). Their total phosphorylase values were 40–50% lower than ours and were probably due to an underestimate of phosphorylase *b* caused by an inadequate ATP concentration (3.5 mM) causing submaximal conversion of phosphorylase *b* into form *a* by phosphorylase *b* kinase. A final ATP concentration of at least 12 mM is required for liver homogenates (Gain, 1976).

Ballard & Oliver (1963) and Devos & Hers (1974) expressed their results as units/g wet wt. of liver. Our results, calculated on that basis, showed a very good agreement with the total synthase values of Ballard & Oliver (1963), who, interestingly, measured the UDP formed which would be unaffected by glycogenolytic interference. Devos & Hers (1974), however, measured the ^{14}C -labelled glycogen and did not wash the foetal liver. They assayed the synthase at 20°C and, on the basis of their temperature coefficient, our results should have been approximately 50% higher than theirs; in fact they were on average approx. 600–700% higher. They used Sephadex G-25 filtrates of the liver homogenates, and this could also have contributed to the lower activities. Despite this, the developmental pattern they observed for total synthase was similar to the present one. Their total phosphorylase activities were on average 15–20% of ours, which, in turn, were more than twice the values that could be accounted for by the temperature difference of the assays.

The developmental patterns of the total synthase and total phosphorylase were almost identical and this would make little sense in terms of glycogen storage if consideration were not given to the physiologically active forms of the enzymes. The fraction of synthase in the active form (Fig. 3) shows a very good correlation with the synthesis of glycogen in the liver. Devos & Hers (1974) showed a similar pattern although their values were somewhat higher. Schwartz & Rall (1973) reported values up to five times the present ones, but they assayed synthase *I* in the absence of Na_2SO_4 which would result in a significant contribution of the *D* form to the activity measured under the *I* assay conditions. Their pattern showed the highest percentage activity at day 19 although they had no values for day 20.

The fraction of phosphorylase in the active form did not correlate with the mobilization of liver glycogen. The significant fall from day 18 to day 19 cannot be explained unless there is some interaction between synthase *I* and phosphorylase *a*. There was no evidence of a 'switch-on' mechanism triggering glycogenolysis at birth. Devos & Hers (1974) found the fraction of phosphorylase in the active form was two to three times higher and at day 21 was 100%. These high values would arise from killing both the mother and foetus by decapitation, which they showed gave raised phosphorylase *a* values compared with foetuses

taken from an anaesthetized mother. The percentage of phosphorylase in the active form reported by Schwartz & Rall (1973) was totally unrealistic in that 92% was active at day 17 and this fell over the remainder of gestation to 53% at birth. These results would arise partly from the inadequate assay of phosphorylase *b* as mentioned above. In terms of phosphorylase *a* activity/g wet wt. of liver our results agreed very closely with those of Ballard & Oliver (1963), who measured only the *a* form.

Glycogen synthase and phosphorylase appear in parallel in the developing liver (Figs. 1 and 2), which would suggest that a common stimulus may be responsible for initiation of their synthesis. In the case of phosphorylase, there appears to be a second stimulus after day 19 causing activation of the enzyme by conversion of phosphorylase *b* into form *a*. This effect was not so obvious for synthase in that the active form appeared in parallel with the total activity.

The most noteworthy finding in the present study was the interrelationship between the active forms of the two enzymes in relation to the glycogen content of the liver (Fig. 4), particularly as phosphorylase *a* alone did not correlate with glycogen deposition and mobilization. The absolute value of the ratio of the two active enzymes is determined by the methods used to assay the enzymes and is not of major importance. Rather, it is the increases or decreases in the ratio that determine the net glycogen content of the liver.

This model suggests that there is continual turnover of glycogen in the liver. Devos & Hers (1974) estimated that their measured activities of synthase would only just account for the synthesis of glycogen *in vivo* during gestation, assuming that there was no turnover of glycogen. While recognizing that there are pitfalls in extrapolating activities *in vitro* to the situation *in vivo*, their calculation applied to our results produced a 10 times greater rate of glycogen synthesis. The foetus could produce a calculated 77 mg of glycogen from day 19 to day 20. In fact, the glycogen content increased by only 10 mg, and therefore a high rate of glycogen turnover could be accommodated by our measured activities. The studies of Birch *et al.* (1974) on the structure of glycogen in adult liver also support the concept of a continuous turnover of glycogen.

Devos & Hers (1974) labelled the glycogen in liver of 20-day-old foetuses by injecting $[6\text{-}^3\text{H}]\text{glucose}$ into the mother. They found the same amount of label at 6 and 42 h after injection. From this, they claimed that there was no turnover of glycogen. However, at day 20 there is rapid formation of liver glycogen and the label would quickly become buried under subsequently added non-labelled glucose molecules. More important though, these workers showed that amylase digestion of this glycogen at 42 h resulted in a parallel loss of labelled and unlabelled glucose.

Further, the outer chains of the glycogen molecule had 70% of the specific activity of the label in the inner chains. This would suggest some cycling of the labelled glucose to keep it distributed throughout the glycogen molecule.

If glycogen turnover was operative by a cycle between phosphorylase *a* and synthase *I*, there would be a ratio between the two enzymes at which no net synthesis or degradation would occur. Movement of the ratio up or down would result in glycogenolysis or glycogenesis respectively and would provide a sensitive mechanism for the regulation of glucose homeostasis. Such a system would explain why there always appears to be a significant amount of phosphorylase in the active form in the liver even under basal conditions or those of net glycogen synthesis. Also it would explain why active glycogen synthesis in the foetal liver at days 19–21 occurs even though the amount of synthase *I* is not greatly above that of adult liver with a much lower glycogen content. The higher activity of phosphorylase *a* seen in the adult liver would produce an enzyme ratio that prevents net glycogen deposition. This system could also explain why synthase responds sluggishly to hormones, over minutes, in contrast with phosphorylase, which responds in seconds. The present hypothesis requires that only one enzyme should be acutely responsive to hormones. A change in phosphorylase *a* activity would alter the ratio sufficiently to initiate net glycogenolysis or glycogenesis, depending on the hormonal balance.

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