Glycogen Metabolism in the Liver of the Foetal Rat

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1. The glycogen present in the liver of rat foetuses was labelled by injecting a trace amount of [6-³H]glucose into the mother at 19.5 days of gestation. The radioactivity incorporated in the glycogen 4 h after the administration of the label was still present 38 h later. A large proportion of this radioactivity was on the outer chains of the polysaccharide. These results indicate that there is normally almost no glycogen degradation in the foetal liver. In contrast, glycogen breakdown occurs very rapidly in the livers of foetuses whose mother is anaesthetized. 2. Glycogen synthetase is present in the liver at day 16 of gestation at a concentration as high as 30% of that in the adult, but essentially as an inactive (*b*) enzyme. The appearance of synthetase phosphatase between days 18 and 19 corresponds to that of synthetase *a* and to the beginning of glycogen synthesis. From day 19 to 21.5 the amount of synthetase *a* present in the foetal liver is just sufficient to account for the actual rate of glycogen deposition. 3. The content of total phosphorylase in the foetal liver increases continuously from day 16 to birth. However, a precise measurement of the *a* and *b* forms of the enzyme in the liver of non-anaesthetized foetuses is not possible. Taking the rate of glycogenolysis as an appropriate index of phosphorylase activity, we conclude that this enzyme is almost entirely in the inactive form in the foetal liver under normal conditions. 4. The accumulation of glycogen in the liver during late pregnancy may therefore be explained by a relatively slow rate of synthesis and a nearly total absence of degradation.

The accumulation of glycogen in the foetal liver during the last period of the gestation in rats is well documented (Shelley, 1961). The enzymic basis of this deposition is, however, not clear. The recent work on the control of glycogen metabolism in the liver has indicated that the rates of glycogen synthesis and degradation are proportional to the concentration of the active (*a*) forms of glycogen synthetase and of glycogen phosphorylase respectively. This concentration depends on the relative activity of the kinases and phosphatases that control the interconversion of the two forms of synthetase and of phosphorylase. The system is arranged in such a way that synthetase is active only when phosphorylase is inactive, and vice versa (for a review see Hers *et al.*, 1970). It is therefore surprising that, in the foetal liver, phosphorylase was found predominantly in its active form (Novak *et al.*, 1972; Schwartz & Rall, 1973) whereas, as expected from the fact that glycogen accumulates, synthetase was also partially active (Schaub *et al.*, 1972). However, since phosphorylase is rapidly activated when the animals are killed and a liver homogenate is prepared (Stalmans *et al.*, 1974), the actual amount of phosphorylase *a* in the liver *in vivo* can only be measured in anaesthetized animals and if special precautions are taken in the manipulation of the liver.

In the present paper we show that the synthesis of glycogen in the liver of foetal rats parallels the

appearance of synthetase *a*, itself secondary to the appearance of synthetase phosphatase. The amount of synthetase *a* present in the foetal liver is just about sufficient to account for glycogen deposition, if no breakdown occurs. Accordingly the absence of glycogen degradation in the foetal liver was shown by radioisotopic experiments. Although the measurement of phosphorylase *a* activity *in vivo* under normal conditions is not possible, the conclusion is reached that phosphorylase is mostly inactive during foetal life.

Materials and Methods

Chemicals

UDP-glucose, D-glucose 6-phosphate, ATP, AMP, cyclic AMP, histone (type II-A from calf thymus) and shellfish glycogen (type II) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. Shellfish glycogen was further purified by insolubilization of impurities first at pH 3 and then in the presence of 0.25M-K₂HPO₄, and dialysed. Alpha particulate glycogen was prepared from rabbit liver as described by De Wulf *et al.* (1970). Glucagon was obtained from Novo Industri A/S, Copenhagen, Denmark. 6-*N*-2'-*O*-Dibutyryl adenosine 3':5'-cyclic monophosphate and NAD⁺ were purchased from Boehringer und Soehne G.m.b.H., Mannheim, Germany. UDP-[U-¹⁴C]glucose and [6-³H]glucose were obtained

from The Radiochemical Centre, Amersham, Bucks., U.K. [γ - ^{32}P]ATP was prepared by the method of Glynn & Chappell (1964) modified by Walsh *et al.* (1971). Sephadex G-25 (medium size) was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Pentobarbital sodium was obtained from Siegfried S.A., Zofingue, Switzerland.

Enzymes

Glucose oxidase, peroxidase and β -amylase (from barley, type II) were obtained from Sigma. Takamine diazyme powder, from Miles Chemical Co., Clifton, N.J., U.S.A., was separated from contaminating glucose by filtration on a Sephadex G-25 column. UDP-glucose dehydrogenase was purchased from Boehringer. Liver phosphorylase *a* was purified 200-fold from dog liver by the method of Appleman *et al.* (1966) with the following modifications: the liver was perfused *in situ* with 0.1M-NaF and homogenized in the same medium; the digestion with α -amylase was omitted.

Handling of animals and of their livers

The rats used for the experiments were of the Wistar strain. Pregnant mothers weighing about 250g were dated by knowing the time of fertilization ($\pm 6\text{h}$) by the presence of a copulation plug. The foetuses were delivered by caesarian section either after decapitation of the mother or under anaesthesia. Anaesthesia of the mother and foetuses was induced by intraperitoneal administration of 7.5mg of pentobarbital/100g body wt. to the mother. When they were non-anaesthetized, the foetuses were decapitated. Their livers were either rapidly removed and homogenized, or quick-frozen as described by Wollenberger *et al.* (1960) and stored at -20°C until processed. A homogenate of one or several livers was prepared in a motor-driven Potter & Elvehjem (1936) Teflon-glass homogenizer with 5 or 10 vol. of ice-cold 0.1M-glycylglycine buffer adjusted to pH 7.4 with 1M-NaOH; when the activity of phosphorylase had to be measured, 0.1M-NaF was also present in the homogenization mixture. An extract was obtained by centrifuging the homogenate for 10min at 8000g and discarding the sediment; 2ml of the extract was filtered through a Sephadex G-25 column (10ml of gel) and a protein fraction (maximally 2.5ml) free of small molecules was collected. A 1.5-fold dilution usually resulted from the filtration procedure.

Determination of metabolites

Glycogen was isolated from tissues and measured after conversion into glucose by Takamine diazyme as described by Hue & Hers (1972). In the livers that were very low in glycogen, the polysaccharide was

measured by the difference in glucose content of a liver homogenate before and after hydrolysis by Takamine diazyme. [^3H]Glycogen was measured in a Packard Tri-Carb liquid-scintillation spectrometer after previous hydrolysis to glucose by Takamine diazyme, by using the scintillation mixture of Patterson & Greene (1965). UDP-glucose was determined in extracts of freeze-clamped liver by the reduction of NAD^+ under the action of UDP-glucose dehydrogenase as described by De Wulf & Hers (1967).

Enzyme assays

Glycogen synthetase *a* was measured in the presence of 10mM- Na_2SO_4 , phosphorylase *a* in the presence of 0.5mM-caffeine and total phosphorylase after complete activation by purified phosphorylase kinase as described by Stalmans *et al.* (1974). For the determination of total glycogen synthetase (synthetase *a* + synthetase *b*) 0.5 μmol of UDP- ^{14}C glucose (30000 c.p.m.), 0.5mg of glycogen, 0.5 μmol of glucose 6-phosphate, 0.05 μmol of EDTA and 5 μmol of glycylglycine buffer adjusted to pH 8.5 with 1M-NaOH were incubated at 20°C for 10 and 20min with 10 μl of a 10% (w/v) homogenate of liver in a total volume of 0.11ml. Radioactive glycogen was isolated and counted as in the determination of synthetase *a* activity. One unit of glycogen synthetase or of glycogen phosphorylase activity is the amount of enzyme that converts 1 μmol of substrate/min in the conditions of the assay.

The activity of synthetase phosphatase was measured by incubating 0.1 unit of liver synthetase *b* purified as described by Stalmans (1969), 20mg of particulate glycogen, 3 μmol of AMP, 5 μmol of magnesium acetate, 25 μmol of 2-mercaptoethanol, 100 μmol of glycylglycine buffer, pH 7.4, and 0.2ml of a 10% (w/v) liver homogenate at 20°C in a final volume of 1ml. At various incubation times up to 120min, 10 μl portions of the mixture were taken for synthetase *a* determination. Synthetase kinase was measured as the rate of inactivation of synthetase *a* in the presence of ATP and Mg^{2+} . For doing so, synthetase was first activated by incubation of a liver extract, previously filtered on Sephadex G-25, in the presence of 5mM- $(\text{NH}_4)_2\text{SO}_4$ for 1h at 20°C . The filtrate, at a final concentration of 10%, was then further incubated at 20°C in the presence of 20mM-NaF, 6mM-ATP, 10mM-magnesium acetate and of various amounts of cyclic AMP. Synthetase *a* activity was then measured in 10 μl samples taken immediately after the addition of the reagents and 5–40min later.

For the determination of phosphorylase phosphatase, 1 unit of purified phosphorylase *a*, 5mg of particulate glycogen, 100 μmol of glycylglycine buffer, pH 7.4, and 0.8ml of a 7% liver extract previously filtered on Sephadex G-25 were incubated in a final volume of 1ml at 20°C . Phosphorylase *a* activity was

measured at various times of incubation in 50 μ l samples. Phosphorylase kinase was measured by the activation, in the presence of ATP and Mg^{2+} , of purified muscle phosphorylase *b*, prepared as described by Fischer & Krebs (1962). One unit of phosphorylase *b*, 6 μ mol of ATP, 10 μ mol of magnesium acetate, 20 μ mol of NaF, 0.1 μ mol of cyclic AMP, 100 μ mol of glycylglycine buffer, pH 7.4, and 0.4 ml of a 20% (w/v) liver homogenate were incubated in 1 ml at 20°C. Phosphorylase *a* activity was measured in 50 μ l samples. The velocity of the various kinases and phosphatases under study tended to slow down when more than 50% of the substrate enzyme was converted. All measurements were made on the linear part of the reaction. Protein kinase activity was determined by method B of Reimann *et al.* (1971) with histone as substrate.

Results

Synthesis and degradation of glycogen in the foetal liver

The concentration of glycogen in the foetal liver at various ages of gestation is shown in Fig. 1. The values obtained in foetuses collected directly after decapitation of the mother are in good agreement with those previously published by several groups of workers

Fig. 1. Glycogen concentration in the liver of foetal rats

In one series of experiments (●) the mother was killed by decapitation and the livers of the foetuses were collected as soon as possible for glycogen determination. In other experiments the mother was anaesthetized with pentobarbital and the foetuses were collected by laparotomy performed 80 (▲) or 100 min (■) afterwards. B = Birth. At days 16–18 the livers from all foetuses of the same litter were homogenized together. From day 19 values shown are means $\pm$ S.E.M. with the numbers of determinations in parentheses.

(Shelley, 1961; Ballard & Oliver, 1963; Avdalovic *et al.*, 1970). Between day 19 and day 21.5, 71.3 mg of polysaccharide was deposited in 1 g of liver, the mean rate of synthesis therefore being 0.11 μ mol of glucose/min per g of liver. It is also shown in Fig. 1 that when the mother was anaesthetized, there was a rapid degradation of glycogen in the foetal livers. The rate of this degradation exceeded 2 μ mol of glucose/min per g of liver.

To estimate the rate of glycogen breakdown in the liver of non-anaesthetized foetuses, the polysaccharide was made radioactive by injecting labelled glucose into the mother at day 19.5 of gestation. For this, 20 μ Ci (10^7 c.p.m. in 30 μ g) of [6- 3 H]glucose was injected either by a single intraperitoneal injection or by two successive subcutaneous injections at a 1 h interval. The animals were killed at various times after the injection and the radioactive glycogen was measured, in the maternal and in each of the foetal livers. As a rule, a greater dispersion of the results was observed when the glucose had been given intraperitoneally than subcutaneously. However, as shown in Fig. 2, there was no significant difference between the radioactivity of glycogen present in the liver shortly after the administration of the label (4–5 h) and much later (40–42 h). Approx. 10 000 c.p.m., i.e. 0.1% of the dose administered, was present in the liver glycogen in all foetuses. A small amount of radioactivity was also incorporated in the glycogen of the maternal liver, particularly when the label had been administered by subcutaneous injections. Most of this radioactivity had disappeared 38 h later.

The radioactive glucose units incorporated into glycogen at an early stage of glycogen deposition (19.5 days) could have been covered by unlabelled units on the periphery of the molecule, when a larger amount of glycogen was synthesized; the degradation of these unlabelled units would not be noticed in our experiments. This is why we have degraded by β -amylase the glycogen collected at 42 h after the administration of radioactive glucose. As indicated in Fig. 3, there was no large difference between the percentage of unlabelled glucose and of labelled glucose taken out by the enzyme. Calculation indicates that the specific radioactivity of the outer chains of the glycogen was about 70% of that of the inner core.

Glycogen synthetase and its converter enzymes

Total synthetase. Fig. 4 shows the total activity of glycogen synthetase (synthetase *a* + synthetase *b*) in the developing liver. As soon as day 16, this activity was at least 20–30% of that found in the adult liver. It increased in the following days, being already close to the adult value on the 18th day. It still doubled soon after birth, and progressively returned to the adult value during the first weeks of life.

Fig. 2. Amount of glycogen (open bars) and of radioactivity in glycogen (filled bars) in the liver of fetuses (F) and of their mother (M) at various times after administration of $20 \mu\text{Ci}$ (10^7 c.p.m.) of $[6\text{-}^3\text{H}]$ glucose to the mother

The labelled glucose was administered either by a single intraperitoneal (I.P.) injection or by two subcutaneous (S.C.) injections at a 1 h interval at 19.5 days of gestation. The values for the glycogen of the mother are for an amount of liver equal to the weight of a foetal liver at the time of injection (180 mg). For the fetuses values shown are means $\pm$ S.E.M. for one litter.

Fig. 3. β -Amylolytic of the foetal glycogen, collected 42 h after the administration of $[6\text{-}^3\text{H}]$ glucose to the mother $[6\text{-}^3\text{H}]$ Glycogen (100 mg, 45000 c.p.m.) was dissolved in 5 ml of 10 mM-sodium acetate buffer, pH 4.8, containing $30 \mu\text{g}$ of β -amylase. The mixture was dialysed at 20°C against the same buffer. Maltose formed ($\bullet$) and ^3H liberated (Δ) were measured at different times in the external fluid.

Fig. 4. Activity of total (a+b) glycogen synthetase in the rat liver during the perinatal period

Before day 19 each point represents a pool of several livers from the same litter. The adult value is the mean of 24 determinations $\pm$ S.E.M. B = Birth.

Active synthetase and UDP-glucose concentration. Fig. 5 shows the concentration of the active form of the enzyme. This was close to zero at day 16 and then increased slowly at day 17 and much more rapidly at

Fig. 5. Synthesetase *a* activity in the liver of foetal rats

Before day 19 each point represents a pool of several livers from the same litter. From day 19 values shown are means $\pm$ S.E.M., with the numbers of determinations in parentheses. B = Birth.

Fig. 6. Double-reciprocal plot showing the effect of the concentration of UDP-glucose on the activity of synthesetase *a*

The Sephadex filtrate of the livers of several 20-day foetuses was incubated at 20°C for 2h in the presence of 5 mM-(NH₄)₂SO₄ to allow full activation of glycogen synthetase. The assays were then performed in the presence of 10 mM-glucose 6-phosphate (○) or of 5 mM-P_i (●). The final concentration of (NH₄)₂SO₄ was 0.5 mM.

days 18 and 19. A maximum was reached at day 21 and a fall occurred at birth. Throughout, synthesetase *a* never exceeded 20% of the total synthesetase in the liver.

The mean activity of the enzyme between day 19 and day 21.5 of gestation was close to 66 nmol/min per g at 20°C. To calculate how much glycogen this amount of synthesetase *a* could synthesize *in vivo*, it was necessary to know the influence of temperature and of substrate concentration on the activity of the enzyme as well as the concentration of UDP-glucose in the foetal hepatocyte. On various occasions we have observed that the activity at 37°C was 2.5-fold that at 20°C, the temperature at which the assays were performed. The Michaelis constant was measured with a liver extract in which synthesetase had been completely activated by a 2h preincubation at 20°C. A value of 0.15 mM was observed (Fig. 6) in the presence of either 5 mM-P_i or 10 mM-glucose 6-phosphate. This value is close to that of 0.2 mM found by Mersmann & Segal (1967) for the adult rat liver enzyme. The fact that the same *K_m* and *V_{max}* values were observed in the presence of P_i, which inhibits synthesetase *b* (De Wulf *et al.*, 1968), and of glucose 6-phosphate, which activates the same enzyme, indicates that the preparation was entirely in the *a* form. The mean concentration of UDP-glucose measured on seven occasions between days 19 and 21 of gestation was 80 nmol (± 11 ; S.E.M.)/g of liver, a value that is markedly lower than that reported in the liver of adult rats (230 nmol/g, according to Hornbrook *et al.*, 1966). This difference is possibly explained, at least in part,

by the presence in the foetal liver of haematopoietic tissues, in which glycogen metabolism is presumably of minor importance. Taking also into account the intercellular and mitochondrial spaces, we assume a concentration of UDP-glucose in contact with glycogen synthetase close to the *K_m* value (0.15 mM). Since the assays were performed at 0.25 mM-UDP-glucose and at 20°C, the correction factor to be applied to convert synthesetase *a* activity, measured by our assay procedure, into a rate of glycogen synthesis *in vivo* is $2.5 \times 0.8 = 2$.

Synthesetase phosphatase. Liver synthesetase phosphatase activity is strongly inhibited by phosphorylase *a*, except if AMP and Mg²⁺ are added to the assay mixture (Stalmans *et al.*, 1971). It is also influenced by the concentration of its substrate, synthesetase *b*, which, as indicated above, varies according to the gestational age. To determine the actual concentration of the phosphatase in foetal liver we have therefore measured it in the presence of AMP and Mg²⁺ and of a given amount of exogenous synthesetase *b*. As shown in Fig. 7, there was no activity at day 16, a slight activity at days 17 and 18 and a much larger one soon after.

When the activation of endogenous synthesetase of the foetal liver was measured in the absence of AMP and Mg²⁺ but in the presence of sulphate or phosphate, this activation was preceded by the typical

Fig. 7. Synthetase phosphatase activity in the liver of foetal rats

The activity was measured by the conversion of exogenous synthetase *b* into synthetase *a*. Sephadex filtrates obtained from the livers of several foetuses of the same litter were used for each determination. The adult value is the mean of 11 determinations $\pm$ S.E.M. B = Birth.

Fig. 8. Sequential changes in the activities of glycogen phosphorylase and of glycogen synthetase in the foetal liver

A Sephadex filtrate of the liver of a 20-day foetus was incubated at 20°C in the presence of 5 mM-(NH₄)₂SO₄. After 90 min (arrow), ATP (6 mM), magnesium acetate (10 mM), NaF (20 mM) and cyclic AMP (0.5 mM) were added (----) at the final concentrations indicated. ○, Phosphorylase *a*; ●, synthetase *a* activity.

Fig. 9. Effect of cyclic AMP on the activity of synthetase kinase of the newborn rat

The experiment was performed as described in Fig. 8 except that the liver of a 4 h-old rat was used and that the concentration of added cyclic AMP was zero, 40 nM, 80 nM, 0.2 μM and 1 μM, from the upper to the lower curve respectively. The inset shows the excess of synthetase inactivated during the first 10 min as a result of the presence of cyclic AMP at various concentrations.

Fig. 10. Activity of total (a+b) phosphorylase and of phosphorylase a in the liver of rats in the perinatal period

Each point represents one individual value or the mean of the activity observed in several foetuses of the same mother, $\pm$ S.E.M., with the number of determinations in parentheses. For the same age, the same open (phosphorylase a) or filled (total phosphorylase) symbols correspond to the same liver. The foetuses were obtained either under anaesthesia ($\square$, $\blacksquare$) or after decapitation of the mother (other symbols). B = Birth.

Fig. 11. Phosphorylase phosphatase activity in the liver of foetal rats

The substrate for the reaction was purified liver phosphorylase a. Sephadex filtrates obtained from the livers of several foetuses of the same litter were used for each determination. The adult value is the mean of three determinations, $\pm$ S.E.M. B = Birth.

latency (Fig. 8) that is known to occur in Sephadex filtrates of adult mouse livers (De Wulf *et al.*, 1970) and also in adult rats and mice *in vivo* (Stalmans *et al.*, 1974). Here also, the latency corresponds to the inactivation of phosphorylase. This indicates that in the foetal, as in the adult, liver the activation of glycogen synthetase is inhibited by phosphorylase a.

Synthetase kinase. Fig. 8 shows that, when glycogen synthetase has been activated, it can be inactivated by the addition of ATP, Mg^{2+} and cyclic AMP; this type of experiment could be performed from day 19 but, owing to the incomplete activation of the synthetase, was difficult to perform at an earlier date. Synthetase kinase activity can also be measured (at least in muscle) by histone phosphorylation (Schlender *et al.*, 1969; Soderling *et al.*, 1970). By using histone and $[\gamma-^{32}P]ATP$ as substrates, we have measured as early as day 16 an activity of protein kinase, stimulated by cyclic AMP, equal to 60% of the adult value. As indicated in Fig. 9, the activity of synthetase kinase was normally sensitive to cyclic AMP. An apparent K_m of $6 \times 10^{-8} M$ was observed with the liver of a newborn rat, and similar values were observed with foetal livers.

Glycogen phosphorylase and its converter enzymes

Phosphorylase. The activities of total phosphorylase and of phosphorylase a in the foetal liver are shown in Fig. 10. The total activity was only about 15% of the adult value until the 18th day of gestation. It then progressively increased to reach the adult value soon after birth. In the livers of the foetuses collected directly after decapitation of the mother, the activity of phosphorylase a was, as a mean, about 80% of the total. This percentage was markedly lowered by anaesthesia of the mother. The administration of glucagon (5 or 50 μg), or cyclic AMP (100 μg), or dibutyryl cyclic AMP (100 μg) or glucose (10 mg) into the umbilical vein had no significant effect on the activity of phosphorylase a (results not shown).

Phosphorylase phosphatase. Fig. 11 shows the activity of phosphorylase phosphatase as measured on exogenous purified liver phosphorylase a. This activity was less than one-half the adult value up to day 21 and then increased rapidly. The inactivation of endogenous phosphorylase in a liver filtrate has also been studied. Fig. 12 shows that at 16 and 18 days of gestation this inactivation was greatly stimulated by caffeine and to a smaller extent by glucose. The latter effect was amplified during the following days, being at day 21 comparable with that observed by Stalmans *et al.* (1970) in the liver of adult mice.

Phosphorylase kinase. An activity of phosphorylase kinase equal to about 40% of the adult value was observed in the liver of foetal rats at 16 and 17 days of gestation (results not shown).

Fig. 12. Inactivation of endogenous phosphorylase by phosphorylase phosphatase at days 16–21 of gestation

A Sephadex filtrate obtained from the livers of several foetuses from the same litter was incubated at 20°C in the presence of 5 mM-(NH₄)₂SO₄. ○, Control; ●, +0.5% glucose; ▲, +5 mM-caffeine. (a) 16 days; (b) 18 days; (c) 20 days; (d) 21 days.

Discussion

The general conclusion that can be drawn from this work is that the accumulation of glycogen in the liver of rats during late gestation is due to a relatively slow rate of synthesis and a complete absence of degradation. These two aspects will be discussed separately.

Glycogen synthesis and activity of glycogen synthetase

Glycogen synthesis. Between day 19 and day 21.5 of gestation, the foetal liver accumulates glycogen at a rate of 0.11 μmol/min per g of liver. This rate may be considered as slow if compared with those of 0.5–1 μmol/min per g reached in adult mice that have been treated with glucose or prednisolone (De Wulf & Hers, 1968).

Total synthetase. The changes in total synthetase activity that we have observed in the liver during the neonatal period are somewhat similar to those published by Schaub *et al.* (1972) and different from those

described by Ballard & Oliver (1963), who did not report the postnatal increase in activity.

Synthetase a. From the data of Fig. 5, it appears that the mean activity of synthetase *a* between days 19 and 21.5 is 66 munits/g of liver. For calculation of the amount of glycogen that this quantity of enzyme can synthesize *in vivo*, taking into consideration differences in temperature and substrate concentration between the assay system and the conditions *in vivo*, the above value needs to be multiplied by 2, indicating a mean rate of synthesis *in vivo* of 0.13 μmol/min per g of liver. This value would be lowered to 0.106 μmol/min per g if one assumed an intracellular concentration of UDP-glucose of 0.1 mM rather than 0.15 mM. It therefore appears that there is just about enough synthetase *a* in the foetal liver between days 19 and 21.5 of gestation to account for the synthesis of glycogen that occurs during the same period.

Synthetase phosphatase and synthetase kinase. The amount of synthetase *a* and *b* in the liver results from

a balance between the action of the two antagonistic enzymes synthetase kinase and synthetase phosphatase. The enzyme is presumably initially synthesized as a non-phosphorylated protein (synthetase *a*). However, synthetase *b* appears in the foetal liver several days before synthetase *a*; this is in agreement with the earlier appearance of synthetase kinase (day 16 or earlier) than of synthetase phosphatase (day 18). The latter event appears to be what initiates glycogen deposition in the foetal liver.

The activity of synthetase phosphatase in the extracts of adult liver is known to be inhibited by phosphorylase *a* (Stalmans *et al.*, 1971) and this inhibition is a major control mechanism *in vivo* (Stalmans *et al.*, 1974). The latency observed in synthetase activation *in vitro* (Fig. 8) suggests that this control mechanism operates also in the foetal liver and that the concentration of phosphorylase *a* is below the threshold value that is inhibitory for synthetase phosphatase.

Glycogen degradation and phosphorylase activity

Glycogen degradation. We have seen above that the foetal liver contains just enough synthetase *a* to account for the observed synthesis of glycogen, suggesting that no degradation of glycogen occurs in the period under study. This conclusion was confirmed by the experiment in which glycogen was labelled with [6-³H]glucose. Indeed, the label incorporated at day 19.5 was still present 38 h later, whereas about 75% of the radioactivity incorporated in the maternal liver glycogen had disappeared during the same period. It is also noteworthy that the outer chains of the polysaccharide had a specific radioactivity equal to 70% of that of the inner core, indicating that most of the molecules synthesized when the labelled glucose was available were not further enlarged. This indicates that during foetal life some glycogen molecules are built up completely before others are synthesized.

Phosphorylase *a*. The absence of glycogen degradation from the foetal liver, together with the presence of a certain amount of synthetase *a*, suggest that phosphorylase is inactive.

A precise determination of the amount of phosphorylase that is in the active form *in vivo* in non-anaesthetized animals appears almost impossible, since the conversion of form *b* into form *a* occurs very rapidly when the animals are killed and their livers removed. From the data of Fig. 1, it appears that anaesthesia in itself induces a rapid glycogenolysis, presumably through phosphorylase activation. Therefore the amount of phosphorylase *a* measured in the liver of anaesthetized animals, although markedly lower than in the liver of animals collected from a decapitated mother, is presumably still much higher than in non-anaesthetized animals. Taking the rate of

glycogenolysis as an index of phosphorylase *a* concentration one can conclude that phosphorylase is almost entirely in the *b* form in the foetal liver.

The reason why phosphorylase is inactive in the foetal liver is not clear. There is no doubt that the foetal liver contains all the machinery that is required to activate phosphorylase and to induce an intense glycogenolysis, since this situation is observed after decapitation of the mother and under anaesthesia. However, the mechanism by which death or anaesthesia of the mother cause glycogenolysis is unknown. In contrast, glucagon appears unable to activate phosphorylase in the foetal liver, and this may be why the enzyme remains normally inactive. Butcher & Potter (1972) have observed that glucagon fails to elicit in the liver of the foetus *in vivo* the large increase in cyclic AMP content that occurs in the adult liver. Snell & Walker (1973) have also shown that the administration of glucagon to newborn rats at the time of delivery does not initiate glycogenolysis. The non-responsiveness of the foetus to cyclic AMP is perhaps even more surprising, since we have observed normal sensitivity of the protein kinase *in vitro* to the cyclic nucleotide.

Another discrepancy between the conditions *in vivo* and *in vitro* was the inefficiency of a glucose load to cause the inactivation of liver phosphorylase in the foetus, whereas phosphorylase phosphatase was stimulated by glucose in a liver filtrate (Fig. 12). Nevertheless, the stimulation of phosphorylase phosphatase by glucose *in vitro* was markedly less pronounced in the foetal liver, particularly at days 16 to 18, than in the adult liver. Since this glucose effect appears to be mediated by the binding of the hexose to phosphorylase *a* (Stalmans *et al.*, 1972), this result suggests that foetal phosphorylase may be different from the adult enzyme.

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