STUDIES ON THE STRUCTURE AND METABOLISM OF GLYCOGEN IN FOETAL LIVER

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Received 24 July 1974
Revised version received 20 January 1975

1 . Introduction

During rat gestation, glycogen rapidly accumulates in the foetal liver from day 18.5 [1–4] and shows notable variations in size [5,6]. It appears at day 17.5 as small particles and by day 19.5 is present, predominantly, as heavy particles, the proportion of which slowly decreases in the next few days. Glycogen storing and its size variations depend on the existence of glucocorticoïds [1,2,7,8].

During this study, we have determined whether there was a relationship between the size variations and the activity of two enzymes involved in glycogen metabolism, the branching enzyme (EC 2.4.1.18) and the amylo 1,6 glucosidase/oligo 1,4 → 1,4 - glucantransferase (EC 3.2.2.33. EC 2.4.1.25).

2. Materials and methods

Adult female Sherman strain rats were mated for an 18 hr period after which vaginal smears were taken and those animals which had sperm present were considered to be at day 1 of gestation. The rat foetuses used in these experiments were 16.5 – 21.5 days old.

In some experiments the bilateral adrenalectomy of the pregnant females was performed at 14 days of prenancy, the animals drank 1% saline instead of tap water. Some foetuses received subcutaneously an injection of 100 μg in 25 μl of hydrocorticortisone acetate (Roussel, Paris, France) on day 16.5 of gestation.

The livers of foetus were rapidly removed, weighed and prepared for enzyme or glycogen extraction and determinations. The glycogen was extracted by mercuric chloride [9,10] and determined as glucose in the glucose oxidase method [11]. The average mol. wt of the glycogen particles was measured by light scattering [10,12] and the profile of distribution of glycogen according to particle size was determined in sucrose density gradients. Branching enzyme was measured by the method of Brown and Brown [13] and amylo 1,6 glucosidase by the method described by Hers, Verhue and Van Hoof [14].

Proteins were determined by the Biuret method [15] and inorganic phosphate was assayed by the procedure of Fishe and Subbarow [16].

Statistical analyses were performed using the student t-test.

3. Results

Table 1 shows that liver branching enzyme activity increased as the liver glycogen concentration increased from 17.5–21.5 days of gestation. Amylo 1,6 glucosidase activity which was high in 16,5 days-old rat foetuses decreased from 17.5–19.5 days and increased from 19.5–21.5 days. This was associated with a marked increase of average mol. wt of glycogen particles from 17.5–19.5 days and a fall from 19.5–21.5 days.

Fig.1 and 2 compare the amylo 1,6 glucosidase activity and the glycogen size throughout late gestation in the liver from intact foetuses, from foetuses receiving hydrocortisone on day 16.5 of gestation and from foetuses originating in adrenalectomized mother on day 14.5. The branching enzyme activity and the glycogen concentration are presented in table 2.

A marked decrease in amylo 1,6 glucosidase was

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Table 1
The development of glycogen concentration, molecular weight and branching enzyme, amylo 1,6 glucosidase activities during foetal liver

Gestational age (days)	Glycogen mg/g Wet liver	Mol. Wt. ($\times 10^{-6}$)	Amylo 1,6 glucosidase activity $\mu g\ P_i$/min/mg prot.	Branching enzyme activity U/hr/mg prot.
16.5	–	–	0.26 ± 0.03 (10)	17.1 [16.9 – 17.3] (2)
17.5	0.6 ± 0.2 (8)	180 ± 40 (6)	0.25 ± 0.04 (11)	17.1 ± 3.2 (8)
18.5	2.8 ± 0.6 (11)	390 ± 40 (11)	0.15 ± 0.02 (10)	22.9 ± 1.7 (9)
19.5	12.5 ± 0.9 (17)	645 ± 55 (17)	0.10 ± 0.02 (15)	45.2 ± 5.5 (15)
20.5	50.8 ± 3.1 (16)	435 ± 20 (16)	0.25 ± 0.05 (13)	101.2 ± 14.1 (13)
21.5	81.0 ± 7.6 (15)	345 ± 30 (15)	0.56 ± 0.09 (10)	218.5 ± 18.4 (10)

The values are means ± S.e.m.

observed in 17.5 day-old rat foetuses in response to cortisol. In contrast, activity of branching enzyme increased under the influence of cortisol. This was associated with an increase in glycogen concentration and size. The histogram of distribution of glycogen according to particle size was determined in sucrose density gradient : the proportion of low glycogen particles rapidly decreased under the influence of cortisol and the proportion of high glycogen particles increased.

As shown in fig.1, the amylo 1,6 glucosidase activity

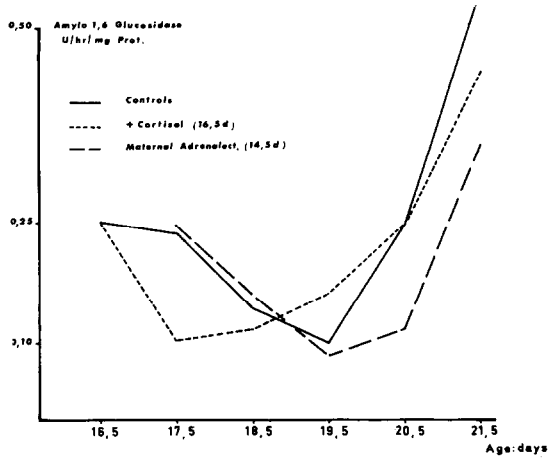

Fig.1. Development of amylo 1,6 glucosidase activity throughout late gestation in the liver. Controls: intact foetuses. + cortisol (16.5 d): foetuses receiving cortisol on day 16.5 of gestation Maternal adrenalect. (14.5 d): foetuses originating in adrenalectomized mother on day 14.5.

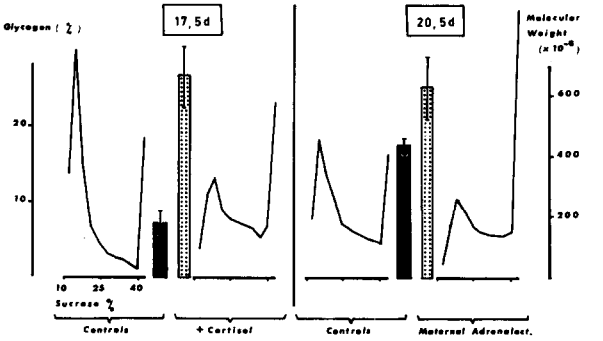

Fig.2. Mol. wt distribution and average mol. wt of glycogen in the liver on day 17.5 and 20.5. The columns indicate average mol. wt ($g \cdot mol^{-1}$). Details are given in Materials and methods.

Table 2
Effects of cortisol and of mother adrenalectomy on liver glycogen concentration, branching enzyme activity of foetal rat

Gestational age (days)		Glycogen mg/g Wet liver	Branching enzyme activity U/hr/mg prot.
17.5	controls	0.6 ± 0.02 (8)	17.1 ± 3.2 (8)
	cortisol	6.4 ± 1.7 (8)[2]	29.6 ± 5.2 (6)[1]
20.5	controls	50.8 ± 3.1 (16)	101.2 ± 14.1 (13)
	adrenalect. mother	49.5 ± 7.7 (7)[ns]	80.7 ± 10.5 (7)[ns]

The values are means ± S.e.m.
[1] Difference significant for $p < 0.05$.
[2] Difference significant for $p < 0.01$.
[ns] = Not significant.

also decreased after maternal adrenalectomy in 20.5 day-old rat foetuses. The branching enzyme activity was slightly modified, and the glycogen concentration became constant, but the average mol. wt of glycogen particles increased. This also was associated with a decrease in high glycogen particles and a marked rise of low glycogen particles.

4. Discussion

As far as the effect of cortisol is concerned, we know that the foetal liver is able to react very rapidly in the presence of corticosteroids [17–19]. This reaction usually manifests itself through an early increase in enzyme activity [20]. As for the enzymes involved in glycogen metabolism, the effect is especially striking with the glycogen synthetase [21,22]; it is confirmed with the branching enzyme. Present findings concerning the amylo 1,6 glucosidase do not make it possible to ascertain its hormonal regulation. The activity of this enzyme is prematurely diminished or maintained at a low rate by glucocorticoids. The decrease in activity noted at day 20.5 after maternal surrenalectomy is probably due to an indirect stimulation of the foetal adrenal glands. For the maternal surrenalectomy increases foetal hypophyseal stimulation and entails an activation of foetal adrenal glands [23,24].

This study especially shows the all important part played by amylo 1,6 glucosidase in glycogen size variations; for there appears during a normal foetal development a relationship between the activity of this enzyme and glycogen mol. wt. This is low when enzyme activity is high, whereas it is higher when the activity is lower. Moreover the activity variations noted under various experimental conditions are followed by size changes in glycogen particles.

Several observations also point out the part played by branching enzyme. For instance it appears that the molecular weight is twice as high at day 20.5 than at day 17.5, whereas amylo 1,6 glucosidase activity remains constant throughout. This is probably related with the increase in branching enzyme activity at day 20.5 which thus diminishes the effects of the debranching enzyme. In the same way, at day 17.5 under the effect of cortisol, it seems as if there were a logical relationship between, on the one hand, the disappearance of a notable proportion of light particles and, on the other hand, both the early increase in the activity of the branching enzyme and the simultaneous decrease in amylo 1,6 glucosidase activity.

Acknowledgements

This work was supported by the Délégation Générale à la Recherche Scientifique et Technique (contrat 7371148).

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