J. Biosci., Vol. 13, Number 2, June 1988, pp. 117–121. © Printed in India.

Glycogen metabolism in human fetal testes

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MS received 25 May 1987; revised 17 September 1987

Abstract. The ontogeny of glycogen synthetase, glycogen Phosphorylase and α-D-glucosidase, enzymes which are associated with glycogen metabolism and glycogen level has been studied in human fetal testes of gestational age ranging from 14-32 weeks. Glycogen synthetase activity reaches the peak value at 17-20 weeks of gestation, thereafter it decreases. α-D-Glucosidase activity increases with the advancement of pregnancy up to 28 weeks of gestation decreasing thereafter very rapidly. Phosphorylase activity remains more or less constant throughout gestation. The maximum increase in glycogen content at early stages of gestation (17-20 weeks) and gradual reduction with the advancement of pregnancy are correlated with histochemical observation by the periodic acid-Schiff technique.

Keywords. Glycogen; glycogen synthetase; glycogen Phosphorylase; α-D-glucosidase; fetus; fetal Leydig cell.

Introduction

Glycogen is the main source of energy in the animal reproductive system. The largest fraction of testicular glycogen is contained in the seminiferous tubule. However, its distribution is not uniform and varies according to the degree of maturity of seminiferous epithelium (Gierke, 1937). It plays an important role in the maturation of germ cells. Intra-tubular glycogen is abundant in the prepubertal stage, diminishes strikingly with the beginning of puberty, and reappears during the period of sexual maturation (Fabbrini *et al.,* 1969). The glycogen level in the seminiferous tubule shows a cyclic behaviour during spermatogenesis at puberty acting as a source of energy in the synthesis of DNA (Re, 1974). The activity of active Phosphorylase, low in prepubertal life (Re *et al.,* 1973), rises during the pubertal phase (Seilicovich and Lloret, 1973) and is very high during spermatogenesis (Mangan and Mainwaring, 1972). In prepubertal testes, in the absence of DNA synthesis, glycogen is not used because of the lack of activation of Phosphorylase (Re, 1974).

Despite all the evidence regarding the existence and function of glycogen in developing mammalian testes little attention has been paid to glycogen metabolism in human fetal testes. The present study was therefore undertaken to measure the activities of glycogen synthetase, α-D-glucosidase and Phosphorylase and the levels of glycogen (histochemically and biochemically) in human fetal testes throughout the gestation period.

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Abbreviation used: PAS, Periodic acid–Schiff.

Materials and methods

All chemicals and reagents used in this study were of analytical grade (E. Merck, Germany and British Drug House, UK). Fine chemicals were purchased from Sigma Chemical Co., St. Louis, Missouri, USA.

Collection of samples

The fetuses were obtained from therapeutic abortions (up to 20 weeks from conception) from different nursing homes and MTP (medical termination of pregnancy) clinics in and around Calcutta. Fetuses above 20 weeks were obtained from stillbirths. The ages of the fetuses were calculated from the mother's menstrual cycle histories and from crown-rump and crown-heel lengths of the fetuses. The method provides data correct to within a week in the majority of cases (Iyengar, 1973). According to gestational age the fetuses were grouped as follows: group I, 14-16 weeks; group II, 17-20 weeks; group III, 21-24 weeks; group IV, 25-28 weeks and group V, 29-32 weeks. Fetuses which showed intrauterine growth retardation were excluded. Both the testes were removed immediately after collection and stored in a freezer (–20°C) for later use.

Human adult testes were obtained from NRS Hospital, Calcutta about 2-4 h after death from (according to available information) healthy persons (25–35 years old) who died in accidents other than testicular injury. The post-mortem stability of the enzymes was frequently checked over a period of 1-8 h and no appreciable changes could be detected.

Enzyme assays

Glycogen synthetase was assayed by the method of Rogers *et al.* (1963). The method of Jauhiainen and Vanha Perttula (1985) was followed for the determination of α-D-glucosidase activity using *p*-nitrophenyl-α-D-glucopyranoside as the substrate. Phosphorylase activity was measured by the method of Freedland *et al.* (1968) in the presence of 0·02 mM adenosine monophosphate (AMP). For the estimation of active Phosphorylase AMP was excluded from the assay system. Glycogen was determined according to the method of Seifter *et al.* (1950). Protein was determined by the method of Lowry *et al.* (1951). The homogenate was kept at 0°–4°C throughout.

For histochemical studies the fetuses were divided into the following groups: early gestation group, 16–20 weeks; mid-gestation group, 21–24 weeks and late gestation group, 25–28 weeks. Both the testes were dissected out as quickly as possible and one of them was fixed in Carnoy's fluid. The presence of glycogen was confirmed by periodic acid Schiff (PAS) staining (Pearse, 1968).

Results

The results presented in table 1 indicate that glycogen synthetase in human fetal testes increases gradually, peaking at 17-20 weeks of gestation and then decreases as gestation progresses. In the adult testes the activity of glycogen synthetase is high, α–D-Glucosidase was found to have a low activity in the early weeks of gestation (14-

Table 1. Age related changes of glycogen metabolizing enzyme activities in human fetal testis.

Gestational age (weeks)	Glycogen (μg/mg tissue)	Glycogen phosphorylase (nmol of P_i liberated/min/mg protein)	Glycogen synthetase (nmol of UDP liberated/min/mg protein)	α-D-Glucosidase (nmol of product liberated/min/mg protein)
14–16 (6)	0·34 ± 0·02	0·98 ± 0·06	1·02 ± 0·09	1·30 ± 0·07
17–20 (5)	0·57 ± 0·04*	1·12 ± 0·07	1·63 ± 0·11*	1·34 ± 0·07
21–24 (5)	0·53 ± 0·04	1·24 ± 0·09	0·49 ± 0·03*	1·56 ± 0·12
25–28 (5)	0·18 ± 0·009*	1·33 ± 0·07	0·48 ± 0·03	1·62 ± 0·10
29–32 (4)	0·11 ± 0·005*	1·42 ± 0·08	0·45 ± 0·04	0·67 ± 0·03*
Adult (3)	0·75 ± 0·05	3·03 ± 0·28	1·26 ± 0·01	0·13 ± 0·01

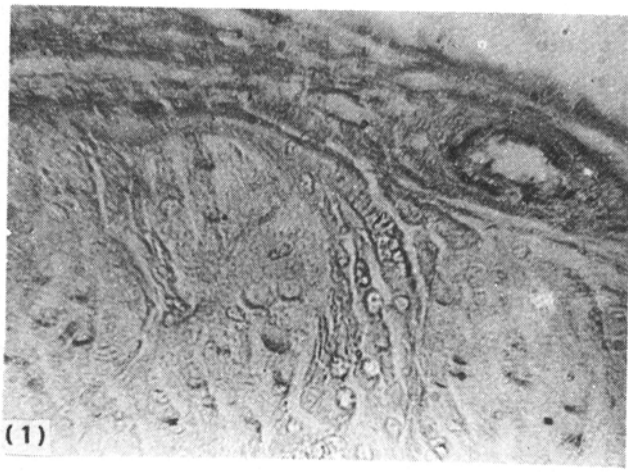
All values are mean ± SE of mean.
Numbers in parentheses indicate the number of cases studied.
*Significant difference (from preceding value), $P < 0·001$ (Student's t test).

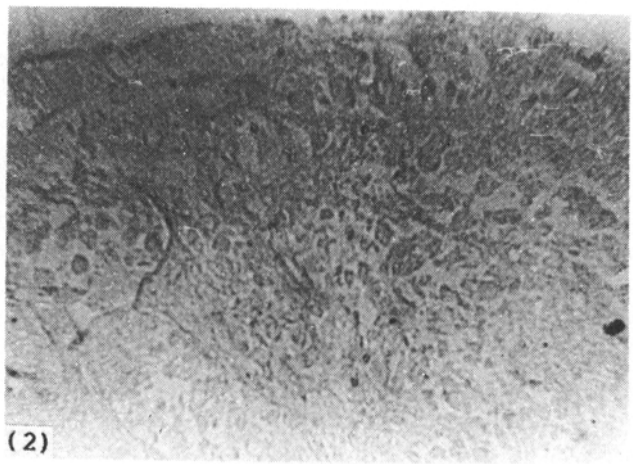
16 weeks); the activity then increases and reaches its maximum level at 25–28 weeks of gestation. However specific activity falls thereafter until the adult level is reached. The total Phosphorylase activity does not alter significantly throughout gestation in human fetal testes and no significant amount of active Phosphorylase could be detected throughout the gestational period. In the adult testes active Phosphorylase activity was detected.

Histochemical examination of human fetal testes (16 weeks gestation) (figure 1) reveals that PAS reaction is little in seminiferous cords and interstitial cells but tunica albuginea reacts more strongly. In mid gestation (21–24 weeks) (figure 2), PAS reaction was found to be less compared to that in the early weeks of gestation. At later stages of gestation (25–28 weeks) (figure 3) PAS reaction was observed to be very faint in seminiferous cords and interstitial cells as well as in tunica albuginea. These observations are in agreement with the biochemical observation that the glycogen content of developing human testes is high at 17–20 weeks of gestation and gradually decreases with the advancement of pregnancy.

Discussion

It is well known that human primordial germ cells contain a large cytoplasmic store of glycogen. In 17–30 day-old human embryos glycogen content remains high in the primordial germ cells (Witschi, 1948). In 44–48-day embryos the primordial germ cells have less glycogen and glycogen also incorporated into the testicular cords. With the formation of tunica albuginea, glycogen deposits are observed among the epithelial cells in both portions of the testicular cords. Fetal Leydig cells also contain PAS positive glycoprotein granules. Gillman (1948) stated that Leydig cells are abundant in fetal life, decrease in postnatal life and increase again at puberty, presumably differentiating from undifferentiated interstitial cell (Hayashi and Harrison, 1971). Shortly after birth the glycogen content of primordial germ cells diminishes (Falin, 1969; Fujimoto *et al.,* 1977). The results presented here (table 1, figures 1–3) reveal that the glycogen content of human fetal testes decreases with increase in gestational age. Histochemically, PAS reaction is little in interstitial cells and seminiferous cords but high in tunica albuginea at early stages of gestation. At





Figures 1–3. PAS reaction in human fetal testis (×400). **1.** I6 weeks. **2.** 22 weeks. 3. 25 weeks.

later stages of gestation PAS reaction is found to be faint in seminiferous cords and interstitial cell as well as in tunica albuginea. The depletion of the glycogen store of human primordial germ cells with progress in pregnancy may reflect its utilization to meet metabolic requirement (Bellve, 1979). The results presented here (table 1) also indicate that the maximum glycogen synthetase activity is also during the period when the glycogen level is maximum *i.e*., at 17–20 weeks of gestation. Not only was total Phosphorylase activity found to be very low, but active Phosphorylase was also not detectable throughout pregnancy (results not shown in the table). Contrary to this, active Phosphorylase activity is very low in prepubertal life (Re *et al.,* 1973) rises during the pubertal phase (Seilicovich and Lloret, 1973) and is very high during spermatogenesis in adult testes indicating the utilisation of glycogen by Phosphorylase. Table 1 also indicates that adult testes have a high activity of active Phosphorylase and this result supports the earlier observations.

α-D-Glucosidase (also known as maltase) catalyzes the cleavage of α-D-glucose residues from poly- and oligosaccharides. This enzyme has some role in glycogen metabolism (Rao *et al.,* 1971). Jauhiainen and Vanha Perttula (1985) have suggested that α-D-glucosidase may function in the digestion of absorbed polysaccharides and glycoprotein as well as in the processing of glycoprotein synthesized by the testis. The high activity of α-D-glucosidase in human fetal testes at 25–28 weeks of gestation suggests that this enzyme is associated with the utilisation of glycogen unlike in adult tissue where glycogen is utilised by Phosphorylase *a.*

In conclusion, it can be stated that glycogen, an important metabolic fuel store, is gradually built up at early stages of development in human fetal testes and its level varies according to the energy needs at different stages of development. α-D-Glucosidase, along with Phosphorylase, is involved in glycogen utilisation of testes during fetal life.

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