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Sex Differences in the Glucose-6-Phosphate Dehydrogenase Activity of the Rat Livers at Various Stages of Development

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출생전후 및 성별로 본 쥐의 간세포에서의 G-6-PD 활성

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摘 要

Pentose phosphate shunt의 첫 작용때 나타나는 효소 glucose-6-phosphate dehydrogenase의 활성을 출생전 및 출생후 연령에 따라서 쥐(white rat)의 간세포를 암수 성별로 spectrophotometer를 써서 측정하였다.

이 활성이 16일 된 배에서 가장 높았고 (54 I.U.), 그 때부터 출생후 13일까지 조금씩 저하되지만 (17 I.U.), 다시 서서히 증가해서 19일에는 53 I.U. 까지 높아졌다.

성별로 본 결과는 일반적으로 수컷보다 암컷에서 G-6-PD의 활성이 약간 높게 나타났다.

INTRODUCTION

The fundamental question of mammalian enzyme development and regulation of enzyme "induction" has been studied intensively during recent years.

The first step in the oxidation of glucose via the pentose phosphate shunt pathway, glucose-6-phosphate dehydrogenase (G-6-PD) catalyses the oxidation of glucose-6-phosphate to 6-phospho-gluconate and this is accompanied by the concomitant reduction of the enzyme nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form ($NADPH_2$).

Recent genetical and biochemical studies on the G-6-PD of human (Tönz and

Rossi 1964), the horse (Trujillo *et al.* 1965), the donkey (Mathai *et al.* 1966) and two species of wild-hares of Europe (Ohno and Poole, 1965) revealed that this enzyme is under the control of a gene located in the X-chromosomes (Kirkman and Hendrickson, 1963).

In man, G-6-PD defficiency results from mutation which develops an acute haemolysis when they receive the synthetic anti-malarial drugs such as primaquine and others (Evans 1963).

It has been evident that the tissue G6PD activities in the rat have significant interaction with sex, age and the time of puberty (Hunter and Hagy 1969). Examination of the livers of suckling rats (1~2 week old) has revealed that the enzyme activity is at about the same level in both sexes. However, hepatic G-6-PD activity is about twice as high in adult females as in males (Hori and Makino 1967). It has also been reported that the hepatic G-6-PD increase was seen on refeeding and after high carbohydrate with adequate protein in rats (Tepperman and Tepperman 1963, Johnson and Sassoon 1967).

In view of the need for a qualitative description of developmental change as a basis for comparative studies, the study of G-6-PD activity of rat livers during prenatal and postnatal periods and of the differences in sex was initiated.

MATERIALS AND METHODS

The 13 pregnant female albino rats (16 to 21 day-old gestation) and 54 young ones, ranging from 0 to 19 days of age, were sacrificed between 8 a.m. and 9 a.m., and the embryo livers were combined according to sex. Sex was determined by examining the gonads after each embryo was dissected, and young animals were handled separately according to sex. The livers were weighed and homogenized in ice. One ml of 0.05M Tris buffer pH 7.5 was used for each 0.1g tissue. Homogenates were centrifuged 17,000 rpm for 10 minutes. at 0° C. Samples were assayed for G-6-PD activity in a Gilfore Model 240 spectrophotometer by reduction of NADP at 340 nm in an assay system containing 0.05M Tris-buffer pH 7.5, 10M glucose-6-phosphate, and 10M NADP (final concentration), supplemented with 203mg of $MgCl_2$ and 50mg of bovine serum albumin. Enzyme activity was expressed in international units per gram of tissue. An international enzyme unit was defined as the amount of enzyme required to catalyze the conversion of 1 micromol of substrate per minute at 25°C.

RESULTS AND DISCUSSION

In Table 1 the G-6-PD activity shows a very high level on the 16 days of emb-

ryo (54.2 units in female) and then decreases to a level in 13 days after birth (17.6 units). There is a significant increase between 13 and 15 days of age and after that continuously increases to the level of 53.2 units at 19 days of age in females. These results show a slight disagreement with Hunter and Hagy who have reported that the livers of the 20 day-old female rat exhibit a lower G-6-PD activity than that of any other age tested. However, 25 day-old rat livers show a higher level of enzyme activity. This little difference in age may be due to a difference in animal strains used and the other environmental factors, such as nutrients, between these two different investigations.

That G-6-PD activity overshoots significantly one day after birth is hard to explain, although, it probably reflects the key position of G-6-PD as the first enzyme in the pentose phosphate shunt.

In general, the G-6-PD activity in female rat livers is slightly higher than in males, even before birth, and it shows a significant increase in female of 19 days of age whichs is not at the stage of puberty (Fig. 1).

This result shows some conflict with previous investigators. Johnson and Sassoon have proven that only in an adult rat liver there is highers enzyme activity in

Table 1. G-6-PD activities in rat livers from 16 days of embryo to 19 days old after birth

Animal		Mean G-6-PD enzyme units	
Age	Number	♂	♀
16-day embryo*	2	54.0	54.2
18 〃	3	37.4	45.0
19 〃	4	38.2	43.1
20 〃	3	34.6	38.3
21 〃	3	34.4	37.4
0-day old**	3	32.1	35.6
1 〃	4	46.2	40.0
2 〃	4	35.4	33.8
3 〃	5	30.8	34.2
4 〃	4	29.7	32.2
5 〃	4	24.2	30.1
6 〃	4	23.2	27.4
7 〃	3	19.6	24.8
9 〃	3	18.5	21.3
10 〃	5	18.3	21.4
12 〃	4	12.8	18.7
13 〃	3	14.2	17.6
15 〃	4	26.6	32.2
16 〃	3	26.8	30.8
19 〃	3	42.2	53.2

*Number of the mothers from which embryo livers were taken out to be put together separately according to the sexes.
**Number of each sex.

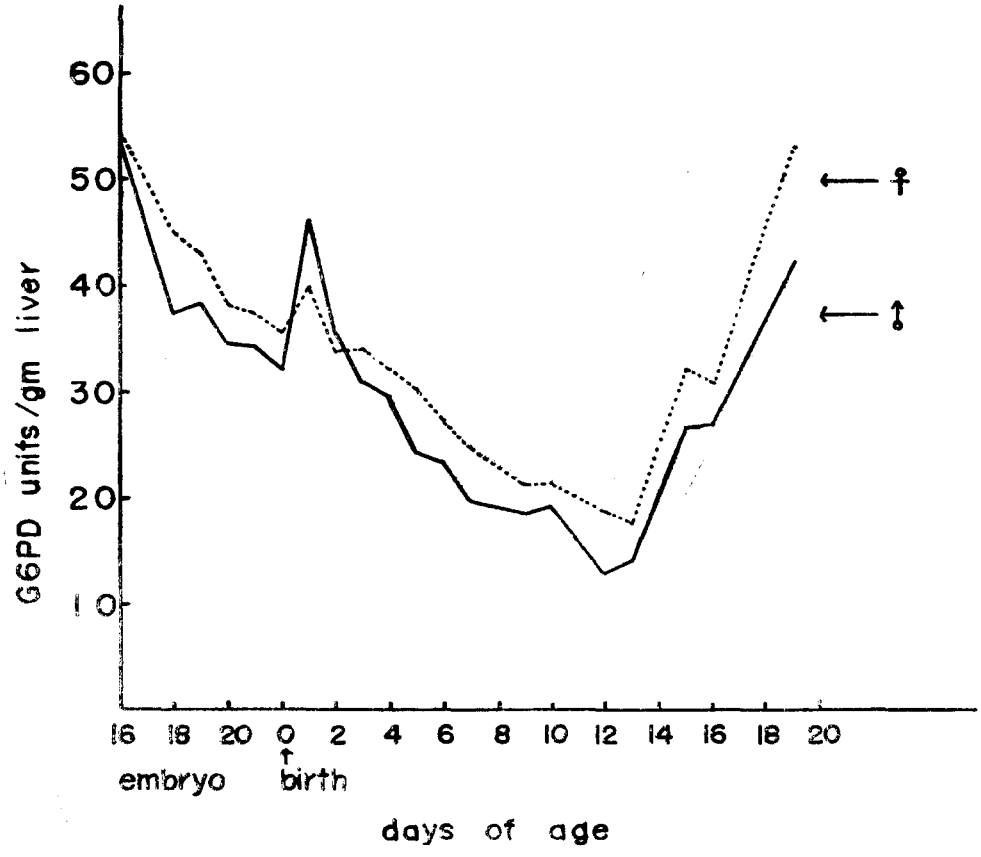

Fig. 1. G-6-PD activities expressed as enzyme unit.

females than in males. Hunter and Hagy also indicated that this might be due to influence of sex hormone in females. Moulton and Barker (1971) reported that the synthesis of additional messenger RNA for G-6-PD leads to 4-fold greater level of enzyme observed after multiple estradiol injections. In regard to control of X chromosome, Lyon (1962) explained that only one X chromosome functions in any somatic cell of female mammals, so that males with only one X chromosome and females with two are functionally equivalent with regard to the gene products of the X chromosome. However, Epstein (1969) reported that the inactivation of the X chromosome does not occur in the mouse oocytes.

And again, even the precise time at which inactivation of the mouse X chromosome occurs still remains to be determined, Lyon (1971) indicated that the inactivation does not occur before the blastocyst stage of development. To confirm the above investigation, the present result should show the same level of enzyme activity in all through the age levels in both sexes.

Even further investigation is necessary to prove whether the certain steroids in certain stage can induce sexual dimorphism in G-6-PD activity in rat livers, it can still conclude that G-6-PD activity change in the course of prenatal development may be due to physiological stimuli rather than the direct action of a gene, such as hormones or nutritional factors.

SUMMARY

The G-6-PD (glucose-6-phosphate dehydrogenase) activity, the first step in the pentose phosphate shunt, of rat livers during prenatal and postnatal development in different sexes was studied.

The enzyme activity is very high (54.2 units) at 16 days of embryo and then decreases to a low level (17.6 units) at 13 days after birth. There are significant increase between 13 and 15 days of age and continuously increases to the level of 53.2 units at 19 days of age.

The G-6-PD activity in female rat livers was slightly higher than in males.

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