Enzyme levels in testis and other tissues of genetically sex-reversed mice

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Summary. The specific activities of G6PD and PGK were measured in the testes, seminal vesicles, and livers of *Sxr/+*,XX mice, their *Sxr/+*,XY littermates and normal mice. While G6PD activity was high in the testes of young normal mice and declined as the testes matured, in the testes of *Sxr/+*,XX mice activity remained high, suggesting a failure of the Sertoli cells to mature normally. The activity of PGK was low in the testes of young normal mice, and increased as the testes matured. The testes of young *Sxr/+*,XX mice had high activity of this enzyme which remained high into adulthood. The high activity in young mice suggests an abnormality in the somatic cells.

The seminal vesicle and liver measurements of G6PD and PGK confirmed that the *Sxr/+*,XX mice were phenotypically normal males except with respect to the testis.

The developmental patterns of both enzymes in testes lacking germinal cells indicate that the maturation of the somatic cells of the normal testis is influenced by the presence of germinal cells.

Introduction

During the development of the testis of the normal mouse, marked changes in the levels of several different enzymes accompany the differentiation of both the germinal cells and the somatic cells (Shen & Lee, 1976). In the period of development before puberty, the germinal cells proliferate and undergo meiosis and spermiogenesis. At the same time, the Sertoli cells form characteristic occluding junctions which are the structural basis of the blood–testis permeability barrier (Flickinger, 1967; Gilula, Fawcett & Aoki, 1976). The Leydig cells also undergo changes which appear to be a de-differentiation followed by a re-differentiation (Gondos, 1977). These developmental changes are accompanied by an increase in the activity of some enzymes such as lactate dehydrogenase and sorbitol dehydrogenase, and a decrease in others such as glucose-6-phosphate dehydrogenase and malate dehydrogenase (Shen & Lee, 1976).

The present study is concerned with developmental changes in the testes of mice without germinal cells. The mice used were of female (XX) chromosomal constitution, but showed a male phenotype due to the presence of an autosomal dominant factor, *sex-reversed* (*Sxr*: Cattanach, Pollard & Hawkes, 1971). Animals of this genotype are phenotypically normal males, but the testes are small and lack germinal cells. The aim of the study was to determine how this germ cell-deficient testis differed from normal in growth and enzyme development, and whether the accessory sex glands were affected.

The two enzymes chosen for analysis show opposite changes in activity during the development of the normal testis. Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) decreases in activity with age in the testes of normal mice (Shen & Lee, 1976) due to a decline in both

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the proportion and enzyme activity of the Sertoli cells in the developing testis (Jones & Andrews, 1978). Phosphoglycerate kinase (PGK, EC 2.7.2.3) increases in activity during the development of the normal testis (Erickson, 1977). There are two isoenzymes present in the testis, PGK A, which is characteristic of somatic tissues, and PGK B, which is found only in the testis, and appears at about the same time as the spermatids (Vandeberg, Cooper & Close, 1976).

The testes of *Sxr/+*,XX mice of various ages were compared to those of normal mice with respect to weight and to activity of the two enzymes, and the enzyme activity of adult seminal vesicles and liver was measured. Enzyme activity measurements were also made on XY mice which carried the *Sxr* factor.

Materials and Methods

Animals

The *Sxr/+*,XX mice used, their *Sxr/+*,XY littermates, and their normal littermates were from stocks maintained at the Unit (by B.M.C.) by crossing *Sxr/+*,XY males to F_1 hybrid (3H1) females derived from the cross C3H/HeH $\times$ 101/H inbred strains. In the studies on young animals the X-linked marker *tabby* (*Ta*) was employed to allow *Sxr/+*,XX individuals to be identified by the *tabby* striping in their coats. The control mice in these studies were 3H1 hybrids. The genotypes of adult animals were determined by the testis weight. It has been shown by Cattanaach (1975) that the *Sxr* factor affects the testis weight: *Sxr/+*,XX animals have a paired testis weight of less than 60 mg, *Sxr/+*,XY animals have a range from 100 to 204 mg and $+/+$,XY animals have testes of 180–240 mg. In our series, animals with testis weights between 180 and 200 mg were not used.

Enzyme assays

Tissues for enzyme activity analysis were extracted in 30 times their own weight of 0.9% (w/v) NaCl + 0.6 mM-EDTA. They were homogenized for 30 sec in an Ultra-Turrax homogenizer. The extracts were then centrifuged for 30 min at 45 000 g, and the supernatants were used in the enzyme assay. Enzymes were assayed by the methods of Bergmeyer (1974). The reaction mixtures were as indicated below.

G6PD: 39 mM-triethanolamine buffer, pH 7.5; 3.9 mM-EDTA; 0.5 mM-NADP (made up at 25 mg/ml in 1% NaHCO₃); and 0.67 mM-glucose-6-phosphate.

PGK: 78.3 mM-triethanolamine buffer, pH 7.6; 0.9 mM-EDTA; 0.2 mM-NADH; 1.1 mM-ATP; 6 mM-3-phosphoglyceric acid; 1.6 mM-MgSO₄; and 10 U glyceraldehyde phosphate dehydrogenase/ml.

All reagents were supplied by Sigma London (Poole, Dorset, U.K.), except EDTA and MgSO₄ which were supplied by B.D.H. (Poole, Dorset, U.K.). The reactions were carried out in a volume of 0.7 ml in a cuvette with a 1 cm light path. The linear change in absorbance at 340 nm was measured in a Pye-Unicam SP 800 spectrophotometer, and the activity was expressed as μ mol NADP reduced/min/mg protein for G6PD, and as μ mol NADH oxidized/min/mg protein for PGK. Protein determinations were by the method of Lowry, Rosebrough, Farr & Randall (1951), as modified by Hartree (1972).

Results

Text-figure 1 shows the increase in testis weight with age in *Sxr/+*,XX mice compared with that of normal 3H1 males. The small testis size of the *Sxr/+*,XX mice was pronounced and the difference in testis weight was apparent by approximately 12 days of age. The testes of these mice reached adult weight by about 30 days, compared with about 40 days in normal mice. The specific activity of G6PD remained high in the testes of *Sxr/+*,XX mice, even when maximum size was reached (Text-fig. 2; Table 1).

Text-fig. 1. Paired testicular weight changes with age in *Sxr/+*,XX mice (+) compared with those of normal 3H1 males (●).

Text-fig. 2. Specific activity of glucose-6-phosphate dehydrogenase in the developing testis of *Sxr/+*,XX mice.

The activity of PGK increased markedly between the ages of 30 and 40 days in normal mice (Text-fig. 3a), but was elevated in the testes of young *Sxr/+*,XX mice and remained high into adulthood (Text-fig. 3b). For mice younger than 30 days, the testis PGK activity for *Sxr/+*,XX mice was both significantly higher and significantly more variable than for normal testes, but after 40 days of age, the values were similar to those for normal adult mice (Table 1).

Text-fig. 3. Specific activity of phosphoglycerate kinase in the developing testes of (a) normal 3H1 mice and (b) *Sxr/+*,XX mice.

Table 1. Mean $\pm$ s.e.m. activity of glucose-6-phosphate dehydrogenase (G6PD) and phosphoglycerate kinase (PGK) in the testes of normal and *Sxr/+*,XX mice (no. in parentheses)

Enzyme	Age	<i>Sxr/+</i> ,XX	<i>+/+</i> ,XX	Significance¶ (Student's <i>t</i> test)
G6PD*	Young (<20 days)	0.134 $\pm$ 0.012 (14)	0.0986 $\pm$ 0.0082 (16)‡	<i>P</i> < 0.017
	Adult (>20 days)	0.146 $\pm$ 0.013 (20)	0.0574 $\pm$ 0.0038 (11)§	<i>P</i> < 0.0001
PGK†	Young (<30 days)	0.352 $\pm$ 0.043 (27)	0.128 $\pm$ 0.010 (32)‡	<i>P</i> < 0.0001
	Adult (>40 days)	0.350 $\pm$ 0.018 (22)	0.438 $\pm$ 0.023 (28)§	<i>P</i> < 0.11

* Values expressed as μ mol NADP reduced/min/mg protein.

† Values expressed as μ mol NADH oxidized/min/mg protein.

‡ 3H1 mice.

§ 3H1 mice and *+/+*,XY littermates of sex-reversed mice.

¶ Probability that the means for *Sxr/+*,XX mice are the same as those for normal mice.

Enzyme activity measurements in various organs of *Sxr/+*,XX mice and their normal and *Sxr/+*,XY littermates are shown in Table 2. Liver was used as a control organ as it is unrelated to sex differentiation. In only one comparison (testicular G6PD) was the difference between the groups of animals significant.

Table 2. Mean $\pm$ s.e.m. (no. in parentheses) activity of glucose-6-phosphate dehydrogenase (G6PD) and phosphoglycerate kinase (PGK) in the testis, seminal vesicle and liver of adult (56–96 days) *Sxr/+*,XX mice and their *Sxr/+*,XY and normal littermates.

Enzyme	Organ	<i>Sxr/+</i> ,XX	<i>Sxr/+</i> ,XY	<i>+/+</i> ,XY
G6PD†	Testis	*0.1283 $\pm$ 0.0148 (11)	0.0792 $\pm$ 0.0096 (8)	0.0546 $\pm$ 0.0052 (7)
	Seminal vesicle	0.0768 $\pm$ 0.0092 (8)	0.0697 $\pm$ 0.0074 (6)	0.0687 $\pm$ 0.0096 (7)
	Liver	0.0117 $\pm$ 0.0028 (9)	0.0080 $\pm$ 0.0021 (6)	0.0067 $\pm$ 0.0014 (7)
PGK‡	Testis	0.3323 $\pm$ 0.0329 (11)	0.4637 $\pm$ 0.0593 (9)	0.4298 $\pm$ 0.0476 (8)
	Seminal vesicle	0.3366 $\pm$ 0.0497 (8)	0.3069 $\pm$ 0.053 (6)	0.2753 $\pm$ 0.046 (7)
	Liver	0.9307 $\pm$ 0.159 (9)	0.6099 $\pm$ 0.159 (6)	0.5242 $\pm$ 0.0859 (7)

* Significantly different from values for *Sxr/+*,XY and *+/+*,XY mice, *P* < 0.0002 (Student's *t* test).

† Expressed as μ mol NADP reduced/min/mg protein.

‡ Expressed as μ mol NADH oxidised/min/mg protein.

Discussion

In considering the differences in testicular enzyme activity between *Sxr/+*,XX and normal mice, it must be borne in mind that the cellular compositions of the testes of the two kinds of animal are vastly different. The testis of *Sxr/+*,XX mice contains gonocytes at birth, but by about 5 days of age there are no germ cells (Cattanach *et al.*, 1971) and all the cells present, Leydig, Sertoli and peritubular cells, are of somatic origin. The normal testis, in contrast, becomes progressively richer in germinal cells as the testis matures. At 6 days, the seminiferous epithelium consists of 84% Sertoli cells and 16% spermatogonia, but by 20 days, there are only 28% Sertoli cells, the rest of the seminiferous epithelium being germinal cells of various stages up to round spermatids (Bellvé *et al.*, 1977). Therefore, our comparisons of enzyme activities in the testes of *Sxr/+*,XX and normal mice are actually comparing cell populations of different origin, morphology and function; but the younger the animals, the more similar are the cell populations which are being compared.

The fact that both G6PD and PGK are coded for by genes on the X chromosome should not be relevant to this study. Although *Sxr/+*,XX mice have two X chromosomes, we are considering only somatic cells in these animals and there is no reason to think that normal X inactivation does not occur in these cells to leave only a single X genetically active. There is likewise no evidence that the activity of X-linked enzymes such as G6PD decreases in germinal cells following the inactivation of the X chromosome at meiosis (Erickson, 1976; Brock, 1977).

Our experiments have shown that the specific activity of G6PD in the testis of *Sxr/+*,XX mice is significantly higher than normal in young and adult mice. We have previously shown that high G6PD activity is characteristic of immature Sertoli cells, but not of either mature Sertoli cells or interstitial cells (Jones & Andrews, 1978). In the *Sxr/+*,XX animals, the adult level of G6PD activity in the testis resembles that in immature testes, suggesting that the Sertoli cells have not matured. This in turn suggests that the decrease in G6PD activity in Sertoli cells of normal mice reflects some interaction with the germinal cells. This conclusion finds support in the histochemical studies of Ito (1966), showing that staining for G6PD around the periphery of the tubules increased after irradiation had caused the disappearance of the germinal cells, and in the work of Kormano, Härkönen & Kontinen (1964) which showed that the G6PD activity of the tubules, as shown by histochemical staining, increased after experimental cryptorchidism in the rat.

Our measurements of PGK activity in testes of normal mice agree with those of Erickson (1977) in showing a large increase in activity during development, but in our mice this increase seemed to come rather earlier (30–40 days), and to be greater in magnitude.

Normal testis has two isoenzymes of PGK: PGK A, which is coded for by a gene on the X chromosome, and PGK B which is coded for by a gene on chromosome 17 (Vandeberg *et al.*, 1976; Cherry & Eicher, 1976). After electrophoresis of whole testis extracts, the bands for PGK A and PGK B are of approximately equal intensity, and Vandeberg *et al.* (1976) considered that PGK B represents the contribution of the later stages of the germinal cells to the total activity, because PGK B is the predominant form in semen. We have found no evidence of any PGK B activity by electrophoresis of testicular extracts of *Sxr/+*,XX mice and it is therefore surprising that the total PGK activity of the testis of adult *Sxr/+*,XX animals is the same as that of the normal testis. However, the level of PGK activity in the immature *Sxr/+*,XX mouse, even at young ages when the cell populations of the testes of normal and *Sxr/+*,XX mice are more similar, is also rather higher than that of the normal testis. This observation indicates that the testis cells in young *Sxr/+*,XX mice are in some way abnormal.

There is similarity in the developmental patterns of testicular G6PD and PGK in *Sxr/+*,XX mice. While in normal mice, testis G6PD activity decreases and the PGK activity increases during the course of maturation in *Sxr/+*,XX mice, the level of both enzymes stays about the same in both young and old mice. This suggests that the development of the somatic cells is

arrested, perhaps because of the lack of germinal cells. It will be important to determine whether these abnormal patterns of development of testis enzymes are peculiar to *Sxr/+*, XX mice, or whether they are also true of the testes of other mice which lack germinal cells, e.g. mice of the *W^v/Wⁿ* genotype for which there is evidence that the morphological development, at least, of the Sertoli cells is normal (Nagano, Suzuki, Kitamura & Matsumoto, 1977).

The measurements of enzyme activity in the seminal vesicles of *Sxr/+*, XY mice, their *Sxr/+*, XY littermates and normal littermates indicated that the lack of germinal cells in the former does not affect the level of these two enzymes in this accessory sex gland. These observations support the view that the *Sxr/+*, XX mice are normal phenotypic males except for their lack of germinal cells.

The measurements of G6PD and PGK in the livers of these three groups of mice show an intriguing similarity of pattern although none of the differences between groups is significant. The livers of the *Sxr/+*, XX mice had the highest mean activity of both enzymes, the *Sxr/+*, XY livers had intermediate, and the normal XY livers had lowest activity. Both these enzymes are part of the glycolytic pathway, and there seems to be no reason to expect any differences in the enzyme activities. Further work, including measurements of normal female livers (+/+ , XX), is required to clarify this point.

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