Evidence for a defective seminiferous tubule barrier in testes of *Tfm* and *Sxr* mice

I. B. Fritz*, Mary F. Lyon† and B. P. Setchell‡§

**Banting and Best Department of Medical Research, University of Toronto, Toronto M5G 1L6, Canada*; †*M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD, U.K.*; and ‡*A.R.C. Institute of Animal Physiology, Babraham, Cambridge, U.K.*

Summary. The competence of the seminiferous tubule barrier was evaluated by determining the sucrose space in isolated testis preparations obtained from adult wild-type mice and from mutants having defects in spermatogenesis. The distribution of [³H]sucrose in testes from wild-type mice was in a space which constituted 18.5% of the [¹⁴C]urea space. The corresponding sucrose space in testes from *Tfm*/Y and *Sxr*/+ sterile mutants was elevated to 57.7% and 52.0%, respectively. Independent approaches to detect an impairment in the seminiferous tubule barrier of testes from *Tfm*/Y mice consisted of estimations of the osmotic barrier to hypertonic LiCl, using histological techniques, and determination of the testis sucrose space *in vivo*. With both approaches, results obtained supported the presence of an impaired barrier in testes of *Tfm*/Y mice. The sucrose space was within normal limits in testes of the other mutants examined, which were defective in spermatogenesis. The results do not provide any evidence for an impaired barrier in testes of mice having *Mo*^{vbr}/Y and *Gy*/Y mutations, or the *t⁶t^w* genotype.

Introduction

The seminiferous tubule barrier, created by integrated tight junctional complexes among adjacent Sertoli cells (Fawcett, 1975), selectively restricts the passage of many molecules from plasma and lymph into the adluminal compartment (Setchell & Waites, 1975). The unique components of tubular fluid in the adluminal compartment create a chemical microenvironment which apparently is required for completion of meiosis and for spermiogenesis. It is generally accepted that Sertoli cells influence and modulate germinal cell development; (1) by forming and maintaining the testis barrier; (2) by producing and secreting particular components such as plasminogen activator, inositol, androgen-binding protein and transferrin; and (3) by implementing the restructuring in the tubule which occurs during various stages of spermatogenesis. Hormonal regulation of spermatogenesis is dependent upon direct actions of FSH and androgens on Sertoli cells and peritubular myoid cells in the tubule, and it appears unlikely that these hormones act directly on germinal cells (for review see Fritz, 1978).

In the mouse a number of mutants and translocations exist in which spermatogenesis is arrested (Lyon & Hawkes, 1970; Cattanach, Pollard & Hawkes, 1971; Searle, Beechey & Evans, 1978; Lyon, Cattanach & Charlton, 1981). In many of these mice, the impairment in spermatogenesis appears to reside within the germinal cells themselves, but possible disturbances in testicular

§ Present address: Department of Animal Sciences, Waite Agricultural Research Institute, Glen Osmond, South Australia 5065, Australia.

somatic cells have not been ruled out. In the androgen-resistant testicular feminization (*Tfm*) mutant, affected *Tfm/Y* genetic males, deficient in androgen-receptor protein, appear as phenotypic females with a small vagina, and small testes but no accessory male organs. In mice chimaeric for *Tfm/Y* $\leftrightarrow$ X/Y, produced by embryo aggregation, *Tfm*-bearing spermatozoa can be produced and can fertilize eggs normally (Lyon, Glenister & Lamoreux, 1975), indicating that spermatozoa do not require the androgen receptor for their development, and suggesting that testicular somatic cells from the X/Y partner are required to permit *Tfm*-bearing germinal cells to develop.

We decided to investigate the competence of the seminiferous tubule barrier in testes obtained from mice which were defective in spermatogenesis.

Materials and Methods

Animals

The animals investigated included *Tfm/Y* and X/Y littermates, and XX *Sxr/+* sex-reversed male mice. In addition, some observations were made on sterile males carrying the X-chromosomal mutant genes viable brindled, *Mo^{br}/Y*, and gyro, *Gy/Y*, and other males sterile due to the presence of two haplotypes of the *t*-complex, *t⁶/t^{wl}*. All these animals were obtained from the M.R.C. colony at Harwell. Additional wild-type mice were obtained from the A.R.C. Institute colony at Babraham.

In-vivo experiments. Mice (8–12 weeks old) were anaesthetized with pentobarbitone sodium (50 mg/kg body wt, intraperitoneally), and both sets of renal blood vessels were ligated. A mixture of [¹⁴C]urea and [³H]sucrose (Radiochemical Centre, Amersham, U.K.) was injected intraperitoneally (0.1 ml/100 g body wt, 15 μ Ci of each isotope/ml). After 1 h, a blood sample was removed from the posterior vena cava, and both testes were removed, decapsulated, and weighed. Testes were minced and incubated in nitric acid (0.1 N, 10 ml/g) for 24 h to extract the markers. Aliquants of the supernatant fluid (0.5 ml) and plasma (0.1 ml) were placed in Fiscint mixture (5 g PPO and 100 g POPOP in a mixture of 1 litre toluene and 0.5 litre Fisons emulsifier), shaken until clear, and counted in a liquid scintillation spectrometer (Searle Analytic 1033) programmed for dual counting of ³H and ¹⁴C.

Counts per min were converted to disintegrations per min of ¹⁴C and ³H, after correcting for quenching, with the use of an analytic 1033 program and the Hewlett-Packard Computer. Volumes of distribution were calculated in μ l/g by dividing the radioactivity per gram of testis by the radioactivity per microlitre of plasma (Setchell, Laurie & Fritz, 1980).

In-vitro experiments. Animals (8–12 weeks old) were killed by a blow on the head, and the testes removed, decapsulated and placed in a Petri dish containing Eagle's minimal essential medium (MEM) (Flow Laboratories, Irvine, U.K.), containing Hank's salt mixture and glutamine, but without NaHCO₃, with bovine serum albumin (Armour, 0.3%) and buffered at pH 7.4 with Hepes (5 mM) and NaOH. Tubules were gently teased apart, but no attempt was made to free the tubules of interstitial cells. Portions (2–30 mg wet wt) were placed in 10 ml Ehrlenmeyer flasks containing 1 or 2 ml of the same medium, but with 0.7 μ Ci/ml each of [³H]sucrose and [¹⁴C]urea. Parafilm-sealed flasks were incubated for 1 h at 32°C in a shaking water bath. At the end of the incubation, the contents of each flask were tipped onto a Millipore filter (0.45 μ m pore) attached to a vacuum flask. As soon as the excess medium had drained off the tissue, the vacuum was disconnected and the tissue transferred to tared vials for weighing. Dilute nitric acid (0.1 N, 1.0 ml) was added, mixed and left to stand at room temperature for 24 h to extract the radioactivity. Scintillation fluid was added as described for the in-vivo experiments, and radioactivity was determined. The volumes of distribution were calculated as in the in-vivo experiments, but using the values for incubation medium instead of those of plasma. A correction for adherent medium was made by obtaining a 'time zero reading'. The apparent [³H]sucrose space in tissue exposed only for seconds to the radioactive medium was assumed to provide a measure of adherent medium, and was subtracted as a blank from the amounts measured in tissues incubated for 60 min.

In other experiments designed to investigate the integrity of the seminiferous tubule barrier by its capacity to resist osmotic shock pressure gradients, we employed the techniques of Gilula, Fawcett & Aoki (1976). Whole decapsulated testes from 7–8 month-old *Tfm/Y* mice, or portions of testes from wild-type mice of similar age, were immersed in 0.75 M-LiCl for various periods of time before being fixed in 4% glutaraldehyde in buffered 0.1 M-collidine containing 25 mM-CaCl₂. Material was subsequently prepared for embedding in paraffin wax. Sections (3–4 µm) were cut and stained with haematoxylin and eosin.

Mean values were compared by Student's *t* tests.

Results

The sucrose space determined in isolated preparations from testes of *Tfm/Y* mice was 212% greater than that in corresponding preparations from testes of wild-type mice ($P < 0.001$), and the testis sucrose space determined *in vivo* in *Tfm/Y* mice was also greater than that in testes of wild-type mice ($P < 0.005$) (Table 1).

Using *in-vitro* procedures, we compared the sucrose spaces in testes obtained from mice having mutations associated with various impairments in spermatogenesis. The sucrose space was larger than normal in testes of *Tfm/Y* and *XX Sxr/+* mice ($P < 0.001$) (Table 2). In contrast, the sucrose space in testes of *Mo^{br}/Y* or *t⁶/t^{wl}* mice was not detectably different from that in testes of wild-type mice ($P > 0.05$) (Table 2).

In preliminary experiments on two *Gy/Y* mice, we observed that the testis sucrose space determined *in vivo* was within the normal range (17.8% and 20.9% of the urea space), suggesting the absence of impairment of the barrier in *Gy/Y* mice.

To assess the integrity of the barrier in testes of *Tfm/Y* mice by an independent method, we employed the procedures of Gilula *et al.* (1976). Testes from wild-type mice and *Tfm/Y* mice were immersed in 0.75 M-LiCl for 0, 1, 2 or 3 min before fixation in buffered glutaraldehyde. Microscopic

Table 1. The volumes of distribution *in vivo* and *in vitro* of [¹⁴C]urea and [³H]sucrose in testes of 8–12-week-old wild-type and *Tfm* mice

	Source of testis	No. of observations	Urea space (µl/g testis)	Sucrose space (% of urea space)
<i>In vivo</i>	Wild-type	5	691 ± 17	16.3 ± 0.7
	<i>Tfm</i>	6	733 ± 24	38.9 ± 2.8
<i>In vitro</i>	Wild-type	67	800 ± 21	18.5 ± 0.9
	<i>Tfm</i>	20	819 ± 32	57.7 ± 1.9

Values are mean ± s.e.m.

Table 2. The *in-vitro* sucrose space in testes of 8–12-week-old mice

Source of testis	No. of observations	Sucrose space (as % of urea space)
Wild-type	67	18.5 ± 0.9
<i>Tfm/Y</i>	20	57.7 ± 1.9
<i>Sxr/+</i>	13	52.0 ± 2.4
<i>Mo^{br}/Y</i>	6	15.3 ± 1.2
<i>t⁶/t^{wl}</i>	9	20.5 ± 1.0

Values are mean ± s.e.m.

examination of the sectioned tubules from testes of wild-type mice indicated that cells in the adluminal compartment appeared normal under each of the above conditions. In testes immersed in hypertonic LiCl for 3 min before fixation, cells in the basal compartment were severely shrunken, while those in the adluminal compartment appeared normal (Pl. 1, Figs 1 & 2). These data confirm the observations of Gilula *et al.* (1976). In contrast, microscopic examination of sectioned testes from *Tfm/Y* mice indicated that all cells within tubules immersed for 1 min or longer in 0.75 M-LiCl before fixation were shrunken (Pl. 1, Figs 3 & 4). The shrinkage of germinal cells located in the centre of the tubule as well as those located near the tubule wall in testes of *Tfm/Y* mice is compatible with the absence of a functional seminiferous tubule barrier in these animals. Osmotic shock affected all cells in the seminiferous tubules of testes from *Tfm/Y* mice, but only cells in the basal compartment of seminiferous tubules of testes from wild-type mice (Plate 1). No cellular shrinkage was evident in sections of control testes from wild-type or *Tfm/Y* mice, in which testes were decapsulated while in MEM, and then fixed directly in buffered glutaraldehyde without prior immersion in LiCl (data not shown).

Discussion

Among the mice examined with mutations associated with impairments of spermatogenesis, testes from *Tfm* and *Sxr* mutants had sucrose spaces far greater than normal. In *Tfm/Y* mice the testis is small, and remains cryptorchid. The most advanced germinal cells are primary spermatocytes, with the occasional appearance of spermatids (Lyon & Hawkes, 1970). Testes from these mice are judged to have a deficient seminiferous tubule barrier on the basis of the large space accessible to [³H] sucrose (Table 1). Ordinarily sucrose is excluded from the adluminal compartment of seminiferous tubules (Setchell & Waites, 1975; Table 1). The normal adult testis sucrose space (18.5% of the urea space) is far less than the sucrose space determined in testes of adult *Tfm* mice (57.7%) and XX *Sxr* mice (52.2%) ($P < 0.001$) (Table 2). The seminiferous tubule barrier in normal adult rodents is relatively resistant to osmotic shock (Gilula *et al.*, 1976; Pl. 1, Figs 1 & 2), but the testes from adult *Tfm* mice had no barrier resistant to osmotic shock (Pl. 1, Figs 3 & 4). It is possible that prolonged cryptorchidism has similar effects on barrier function, and that the impaired barrier observed in testes of *Tfm* mice is an indirect consequence of cryptorchidism, or other factors not necessarily directly related to androgen resistance of Sertoli cells in *Tfm* mice.

Similarly, the enlarged sucrose space in testes of *Sxr* mice could have resulted from factors not directly caused by the effects of the mutation on Sertoli cell functions. *Sxr* mice are chromosomal females which carry a genetic factor for sex reversal, resulting in a male phenotype, with normal accessory sex glands (Cattanach *et al.*, 1971). The adult *Sxr* testis has a normal complement of Leydig cells, but contains no germinal cells. H-Y antigen is expressed in *Sxr* mice (Wachtel, 1977).

The diameter of the tubules in testes of *Sxr* adult mice is reduced, associated with the complete absence of germinal cells in these animals. The interstitial space is enlarged, thereby contributing to the large sucrose space observed. The sucrose space in testes of 7-day-old mice was previously reported to be 35.6% by similar in-vitro techniques (Setchell *et al.*, 1980). Tubules in testes from 7-

PLATE 1

Microscopic appearance of mouse testes subjected to osmotic shock by immersion in LiCl.

Figs 1 & 2. Wild-type adult, 8 months of age; LiCl shock for 3 min. The only cells that are shrunken are those in the peripheral portions of the tubule, corresponding to the basal compartment. Fig. 1, $\times 310$; Fig. 2 $\times 780$.

Figs 3 & 4. *Tfm* adult, 7 months of age; LiCl shock for 1 min. Cells from all portions of the testis are shrunken. Fig. 3, $\times 310$; Fig. 4, $\times 780$.

PLATE I

(Facing p. 362)

day-old mice are approximately the same diameter as those in testes from adult *Sxr* mice (unpublished observations). The smaller sucrose space in 7-day-old mouse testes (37%) (Setchell *et al.*, 1980), than in testes from adult *Sxr* mice (52%; Table 2) suggests that additional factors are contributing to the sucrose space determined in *Sxr* testes, perhaps associated with an increased percentage of the testis volume contributed by the interstitium (Chung, Blackburn, Bullock, Santen & Bardin, 1977). In the absence of germinal cells in the adluminal compartment of testes of *Sxr* mice, it is not possible to evaluate the degree of shrinkage of cells in this compartment, and we therefore could not use the LiCl osmotic shock resistance technique to assess the integrity of the barrier in testes of *Sxr* mice, at least with light microscopy. We interpret the data presented to indicate that the seminiferous tubule barrier is probably impaired in testes of *Tfm/Y* mice, and may be impaired in testes of *Sxr/+* mice. On the other hand, experimental results do not provide any evidence for an impaired barrier in testes of mice having *Mo^{ubr}/Y* and *Gy/Y* mutations, or the *t⁶t^{wl}* genotype.

It is possible that the absence of spermatogenesis in patients with Klinefelter's syndrome (XXY) is similar to the impairment in *Sxr* mice, and that a deficiency in the seminiferous tubule barrier is common to both sets of conditions. This may perhaps be correlated with abnormalities in the ultrastructure and oxidative enzyme activity observed in Sertoli cells from testes of *Sxr* mice (Chung, 1974a, b). Until further information is acquired, however, it is not possible to state the extent to which initial impairments in the seminiferous tubule barrier elicit defects in spermatogenesis in the *Tfm/Y* or *Sxr* mutants. The absence of detectable impairments in the barrier in testes of sterile mice having *Mo^{ubr}/Y* and *Gy/Y* mutations, or the *t⁶T^{wl}* genotype, suggests that the arrest in spermatogenesis in these mice is not associated with malfunction of the barrier, but that the impairment resides elsewhere, presumably within the germinal cells.

References

- Cattanach, B.M., Pollard, C.E. & Hawkes, S.G. (1971) Sex reversed mice XX and XO males. *Cytogenetics* **10**, 318–337.
- Chung, K.W. (1974a) Fine structure of Sertoli cells and myoid cells in mice with testicular feminization. *Fert. Steril.* **25**, 325–335.
- Chung, K.W. (1974b) A morphological and histochemical study of Sertoli cells in normal and XX sex reversed mice. *Am. J. Anat.* **139**, 369–375.
- Chung, K.W., Blackburn, W.R., Bullock, L.P., Santen, R.J. & Bardin, C.W. (1977) Leydig cell structure-function relationships in the mouse with XX sex reversal. In *The Testis in Normal and Infertile Men*, pp. 45–55. Eds P. Troen & H. R. Nankin. Raven Press, New York.
- Fawcett, D.W. (1975) The ultrastructure and functions of the Sertoli cell. In *Handbook of Physiology. Male Reproduction*, Vol. V, Section 7, pp. 21–55. Eds R. O. Greep & D. W. Hamilton. Am. Physiol. Soc., Washington, D.C.
- Fritz, I.B. (1978) Sites of action of androgens and follicle stimulating hormone on cells of the seminiferous tubule. In *Biochemical Actions of Hormones*, Vol. V, pp. 249–281. Ed. G. Litwack. Academic Press, New York.
- Gilula, N.B., Fawcett, D.W. & Aoki, A. (1976) The Sertoli cell occluding junctions and gap junctions in mature and developing mammalian testis. *Devl Biol.* **50**, 142–168.
- Lyon, M.F. & Hawkes, S.G. (1970) X-linked gene for testicular feminization in the mouse. *Nature, Lond.* **227**, 1217–1219.
- Lyon, M.F., Glenister, P.H. & Lamoreux, M.L. (1975) Normal spermatozoa from androgen-resistant germ cells of chimaeric mice and the role of androgen in spermatogenesis. *Nature, Lond.* **258**, 620–622.
- Lyon, M.F., Cattanach, B.M. & Charlton, H.M. (1981) Genes affecting sex differentiation in mammals. In *Mechanisms of Sex Differentiation in Animals and Man*, Ch. 9, pp. 329–386. Eds C. R. Austin & R. G. Edwards. Academic Press, London.
- Searle, A.G., Beechey, C.V. & Evans, E.P. (1978) Meiotic effects in chromosomally derived male sterility of mice. *Annls Biol. anim. Biochim. Biophys.* **18**, 391–398.
- Setchell, B.P. & Waites, G.M.H. (1975) The blood–testis barrier. In *Handbook of Physiology: Male Reproduction*. Vol. V, Endocrinology Section, pp. 143–172. Eds R. O. Greep & D. W. Hamilton. Am. Physiol. Soc., Washington, D.C.
- Setchell, B.P., Laurie, M.S. & Fritz, I.B. (1980) Development of the function of the blood–testis barrier in rats and mice. In *Testicular Development, Structure and Function*, Ch. 6, pp. 65–69. Eds E. Steinberger & A. Steinberger. Raven Press, New York.
- Watchel, S.S. (1977) H-Y antigen: genetics and serology. *Immunol. Rev.* **33**, 33–58.

Received 16 July 1982