

Effects of vasectomy on the blood–testis barrier of the hamster

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Summary. The effects of vasectomy on the blood–testis and blood–epididymal barriers to $^3\text{H}_2\text{O}$, [^3H]inulin, and [^{14}C]urea were examined by study of the radioactivity appearing in micropuncture samples of fluids from the seminiferous tubules and cauda epididymidis. By 4 months after vasectomy, there were changes in the blood–seminiferous tubule barrier to [^3H]water and [^{14}C]urea (increased entry) and in the blood–epididymal barrier to [^3H]water and [^3H]inulin (increased entry) and to [^{14}C]urea (decreased entry). These subtle changes could have an impact on spermatogenesis and/or sperm maturation after vasectomy.

Introduction

Current clinical interest in vasectomy has made vasectomy research a dynamic field of investigation in recent years (see Neaves, 1975, for review). Whether or not vasectomy significantly interferes with normal testicular function has been one of the important questions in the area. There are reports of testicular function being seriously altered after vasectomy (Sackler, Weltman, Pandhi & Schwartz, 1973; Alexander, 1973; Kothari & Mishra, 1973), but there is evidence from a variety of primate and non-primate species which supports the concept that, in general, spermatogenesis continues in vasectomized males (Skinner & Rowson, 1968; Paufler & Foote, 1969; Flickinger, 1972; Vare & Bansal, 1973; Howards, Jessee & Johnson, 1975). Even in vasectomized males with persistent spermatogenesis, however, there are often signs of some alteration or impairment of the spermatogenic process (MacMillan, Desjardins, Kirton & Hafs, 1968; Igboeli & Rakha, 1970; Alexander, 1973). After vasovasostomy in men, sperm counts and fertility often remain low (Kar & Phadke, 1975; Schmidt, 1975) and while such low fertility may sometimes be due to inadequate surgical technique (Silber, 1975), it is also possible that vasectomy itself causes changes in normal testicular and epididymal function.

The present study was performed in order to examine the effects of vasectomy on the blood–testis and blood–epididymal barrier. Knowledge of the state of these barriers after vasectomy is important in understanding the spermatogenic process after vasectomy and how vasectomy might affect male infertility even after vasovasostomy.

The methods and some data for the control group of this study have been the subject of a previous paper (Howards, Jessee & Johnson, 1976).

Materials and Methods

Experiments

Mature male hamsters weighing from 100–150 g were maintained in constant–temperature housing with a 14 h light/10 h dark cycle. Treatment animals were bilaterally vasectomized using standard techniques 4 months before experimental use. Care was taken not to involve the vasal

artery and vein during ligation and section of the vas deferens. The scrotal position of the testes after surgery was confirmed. Measurements of testicular length were obtained from all animals after vasectomy and compared to those for a group ($N = 6$) of age-matched intact hamsters. The hamsters were anaesthetized with an intraperitoneal injection of sodium 5-ethyl-5-(1-methyl propyl)-2-thiobarbiturate (Inactin: Byk Guilden Konstanz, Hamburg, Germany) in a dose of 200 mg/kg body weight. All animals were bilaterally nephrectomized. A jugular venous cannula was inserted in all animals for infusion of saline and isotopes. A carotid arterial cannula was installed to allow sampling of arterial blood. The testis and epididymis were approached through a scrotal incision and prepared for micropuncture as previously described (Johnson & Howards, 1975). The collection of seminiferous tubule fluid and fluid from the cauda epididymidis by micropuncture and of blood plasma was as described by Howards *et al.* (1976). Cell-free fluids were obtained by centrifuging samples in a refrigerated (0°C) IEC model B-20 centrifuge at 35 000 g for 15 min.

Utilizing a specially constructed vertical transfer apparatus (Bunton Instrument Co., Rockville, Maryland), 100 nl of the cell-free fluid were transferred under oil to a calibrated volumetric pipette. This measured aliquot was then placed in 10 ml scintillation fluid and counted in a scintillation spectrophotometer (Packard Tri-Carb, Model 3375). All samples were prepared and analysed in duplicate. All animals were infused with one of the three labelled compounds in this study. Infusion protocols were similar to those described by Howards *et al.* (1976) and were as described below.

$^3\text{H}_2\text{O}$. Eight (8) control and five (5) vasectomized animals were infused with 1 mCi $^3\text{H}_2\text{O}$ (sp. act. 1 mCi/g; New England Nuclear, Boston, Massachusetts) over a 3-min period. Micropuncture samples were collected 15 min after infusion and approximately every 15 min thereafter for 100 min.

^3H]Inulin. Six (6) control and four (4) vasectomized animals were infused with 330 μCi [^3H]inulin (sp. act. 240–500 mCi/g; New England Nuclear) in 3 ml of 0.9% (w/v) NaCl solution over a 15-min period. Samples were collected at 30 min after infusion and every 30 min thereafter for 210 min.

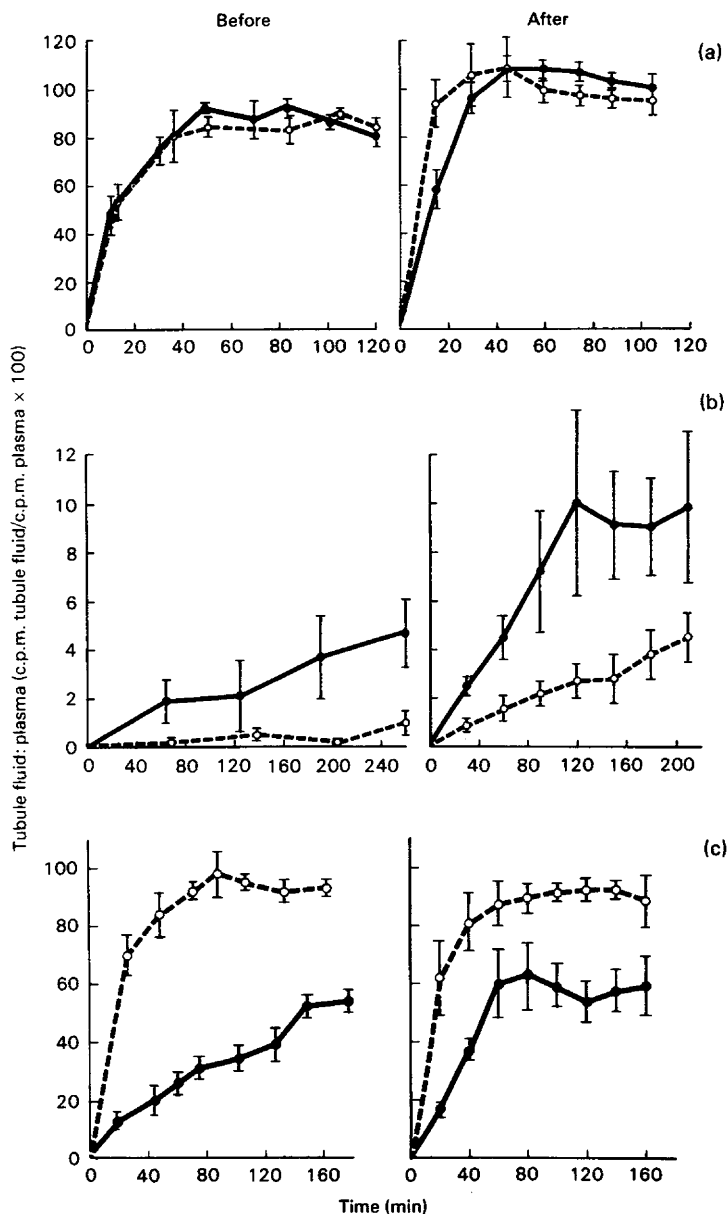
^{14}C]Urea. Six (6) control and four (4) vasectomized animals were infused with 500 μCi [^{14}C]urea (sp. act. 60 mCi/mmol; New England Nuclear) in 0.5 ml 0.9% NaCl solution over a period of 1.5 min. A 'sustaining dose' of 250 μCi [^{14}C]urea in 2 ml 0.9% NaCl was infused throughout the rest of the experiment. Sample collection began 20 min after infusion and was repeated thereafter every 20 min for 160 min.

Data analysis

After subtraction of background counts, the counts per minute (c.p.m.) per unit volume in the tubule fluid were divided by the c.p.m. per unit volume in the plasma. At each time for each compound, the number was multiplied by 100 to give a tubule fluid:plasma ratio that at equilibrium equalled 100 (tubular fluid c.p.m./plasma c.p.m. $\times 100 = 100$). Slope coefficients were calculated for entry for each compound into fluid from the seminiferous tubules and cauda epididymidis of each animal. The slope coefficient ('b' term in the linear regression formula $y = a + bx$) allows a measurement of the initial rate of entry (slope of the line) of each isotope into each tubule fluid. The tubule fluid:plasma data used to calculate slope coefficients were from 0 to 45 min for $^3\text{H}_2\text{O}$, 0 to 200 min for [^3H]inulin, and 0 to 80 min for [^{14}C]urea. Mean plateau values were calculated for each animal during the period from 45 to 105 min for $^3\text{H}_2\text{O}$, 80 to 160 min for [^{14}C]urea, and 120 to 210 min for [^3H]inulin. The mean data for each compound and its entry into fluid of the seminiferous tubules and cauda epididymidis of the vasectomized animals were compared to the blood-testis barrier data of normal animals by the Student's *t* test.

Results

The results for entry of $^3\text{H}_2\text{O}$ into the tubular fluids of intact animals are illustrated in Text-fig. 1(a); this compound entered the most rapidly of the three labelled compounds administered. The mean slope coefficients given in Table 1 indicate that the entry rate for $^3\text{H}_2\text{O}$ into the seminiferous tubule fluid after vasectomy was significantly accelerated ($P < 0.01$) but no



Text-fig. 1. The curves for entry of (a) $^3\text{H}_2\text{O}$, (b) $[^3\text{H}]\text{inulin}$, and (c) $[^{14}\text{C}]\text{urea}$ into the fluid of the seminiferous tubule (●) and in the cauda epididymidis (○) of hamsters before and 4 months after vasectomy. Values are mean \pm s.e.m. for (a) 8 and 5, (b) 6 and 4, and (c) 6 and 4 control and vasectomized animals, respectively.

Table 1. Mean \pm s.e.m. values (no. of observations in parentheses) for entry of labelled compounds into fluids of the seminiferous tubule and cauda epididymidis of hamsters before and after vasectomy

Compound	Seminiferous tubule		Cauda epididymidis	
	Before	After	Before	After
<i>Initial slope coefficients</i>				
$^3\text{H}_2\text{O}$	1.22 ± 0.09 (4)	1.78 ± 0.05 (5)**	1.31 ± 0.11 (4)	1.42 ± 0.11 (5)
[^3H]Inulin	0.02 ± 0.01 (4)	0.08 ± 0.03 (4)	0.00 ± 0.00 (4)	0.02 ± 0.01 (4)*
[^{14}C]Urea	0.37 ± 0.04 (5)	0.84 ± 0.18 (4)*	1.12 ± 0.06 (5)	1.02 ± 0.08 (4)
<i>Plateau values</i>				
$^3\text{H}_2\text{O}$	87.05 ± 2.58 (5)	99.38 ± 2.17 (5)**	87.61 ± 2.72 (4)	96.78 ± 2.19 (5)**
[^3H]Inulin	—	9.57 ± 1.23 (4)	—	—
[^{14}C]Urea	42.50 ± 3.20 (5)	57.98 ± 3.79 (4)**	94.93 ± 1.57 (5)	90.42 ± 1.91 (4)*

Values significantly different from those before vasectomy, * $P < 0.05$, ** $P < 0.01$.

significant effect was demonstrated for entry into the epididymal fluid. The plateau levels for both fluids were significantly increased after vasectomy ($P < 0.01$).

The results for [^3H]inulin (Text-fig. 1b) showed that slope coefficient data for entry of [^3H]inulin into seminiferous tubule fluid were highly variable. The mean slope coefficient was unchanged for the seminiferous tubule values, but significantly increased ($P < 0.05$) for epididymal fluid values after vasectomy (Table 1). Plateau levels could not be compared statistically because only one plateau value for [^3H]inulin could be obtained.

The values for entry of [^{14}C]urea into the tubular fluids are shown in Text-fig. 1(c). The mean slope coefficient was significantly increased ($P < 0.05$) only for entry into seminiferous tubule fluid (Table 1). The change of plateau levels (Table 1) was a significant increase ($P < 0.01$) for seminiferous tubule fluid and a significant decrease for epididymal fluid.

The testicular length before vasectomy (1.85 ± 0.03 cm) was not significantly different from that after vasectomy (1.85 ± 0.05 cm).

Discussion

The blood–testis barrier provides a special environment for the proper development of spermatozoa. Studies of the barrier at the level of the rete testis (Setchell, Voglmayr & Waites, 1969; Setchell & Wallace, 1972; Cooper & Waites, 1975) gave information for the compartment distinct from that of the seminiferous tubules. However, since the barrier's primary site of action is the seminiferous tubule, this is a logical site to examine, and micropuncture of seminiferous tubules *in vivo* is the most direct, physiological method for obtaining fluid from the seminiferous tubules.

Howards *et al.* (1976) demonstrated in the hamster that the blood–epididymal barrier is similar, though not identical to, the blood–testis barrier: fluids in the seminiferous tubule and the cauda epididymidis are easily and equally permeable to $^3\text{H}_2\text{O}$, [^3H]inulin is largely excluded from both ducts and [^{14}C]urea enters epididymal fluid at a significantly greater rate than it enters seminiferous tubule fluid. The present report describes the effects of vasectomy 4 months previously on these characteristics of the hamster blood–testis and blood–epididymal barriers.

The changes in entry of $^3\text{H}_2\text{O}$ were surprising because it was expected that water would be permeable to the ducts freely. The biological significance of these findings is unclear.

The results for entry for [^3H]inulin into seminiferous tubules preclude any firm conclusions but if the apparent increase in [^3H]inulin entry after vasectomy is confirmed, damage to the tubule wall would be implied. The increased entry of [^3H]inulin into the epididymal fluid probably reflects a leakage of the inulin, and presumably other large molecules. Similar changes have been described in the inulin permeability of renal tubule after ureteral obstruction (Lorentz,

Lassiter & Gottschalk, 1972). This leakage could be due to the physical distension of the cauda epididymidis after vasectomy and subsequent damage to the epididymal tubule wall. Whether or not this leak persists when vas occlusion is removed is unknown.

The entry of [^{14}C]urea into cauda epididymal fluid was faster than in seminiferous tubule fluid both before and after vasectomy. The reduction of isotope plateau values in the cauda fluids after vasectomy is difficult to interpret. The increased entry into the seminiferous tubule fluid after vasectomy may not imply a breakdown of a structural barrier. Johnson & Howards (1975, 1976) have shown, in the hamster and guinea-pig, that the increased pressure in the cauda epididymidis after vasectomy does not exist in the seminiferous tubule and breakdown of a structural barrier due to distension is therefore unlikely. Additionally, Neaves (1973) has shown with electron microscopy that the blood-testis barrier to lanthanum is still intact 4 months after vasectomy.

There is some evidence for active transport of [^{14}C]urea out of the hamster seminiferous tubule (Turner & Howards, 1978). The increased [^{14}C]urea entry into the seminiferous tubule in vasectomized hamsters could be due to a decrease in activity of an outward directed urea pump in the epithelium of the tubule rather than to an increase in the permeability of the tubule wall to ^{14}C urea. This subtle change in a function of the seminiferous tubule could be singular and of no real importance, or it could be only one of several small defects previously undetected. Physiological alterations of this kind could have serious effects on sperm development even though the cells continue to be present and of apparently normal morphology.

Physiological modifications not repairing themselves after relief of vas occlusion might be an important factor in the persistent infertility of human males after vasovasostomy.

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