The Effect of Estrogen Administration *In Vivo* on the Elemental Composition of the Intraluminal Fluids of the Seminiferous Tubules, Rete Testis, and Epididymis of the Rat

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The maturation of spermatozoa in the epididymis is dependent upon the presence of androgens. This study examined the effects of androgen suppression by estradiol valerate on the elemental composition of the intraluminal fluids of the testis and epididymis. In the fluid from the caput epididymidis, the concentrations of sodium (106.1 ± 3.4 to 182.8 ± 16.9 mmol/1, $P < 0.01$) and chloride (16.5 ± 2.2 to 79.3 ± 10.8 mmol/1, $P < 0.01$) rose after treatment with estradiol valerate. By contrast, this treatment reduced the concentrations of phosphorus (63.7 ± 1.6 to 47.8 ± 3.2 mmol/1, $P < 0.01$), sulfur (18.4 ± 1.0 to 10.8 ± 1.0 mmol/1, $P < 0.01$), calcium (0.93 ± 0.09 to 0.50 ± 0.07 mmol/1, $P < 0.01$), and magnesium (2.21 ± 0.41 to 0.76 ± 0.16 mmol/1, $P < 0.01$). In the distal cauda epididymidis, the concentration of chloride rose after treatment with estradiol valerate (24.4 ± 1.7 to 54.9 ± 3.9 mmol/1, $P < 0.01$), but the concentrations of the other measured elements (sodium, potassium, phosphorus, calcium, magnesium, and sulfur) were not altered by estrogen treatment. In rete testis fluid the concentration of phosphorus fell (2.00 ± 0.30 to 0.67 ± 0.12 mmol/1, $P < 0.01$), while that of calcium rose (0.66 ± 0.15 to 1.55 ± 0.21 mmol/1, $P < 0.01$). Estrogen treatment did not appear to affect the elemental composition of seminiferous tubular fluid or serum. Therefore, estradiol valerate had a marked impact on the elemental composition of luminal fluid only in the caput epididymidis—where sperm maturation is initiated—and a minor effect on that of cauda epididymidal fluid—in which mature spermatozoa are stored.

Key words: epididymis, spermatozoa, estrogen, elements, testis, sodium, seminiferous tubule.

Spermatozoa acquire the capacity for motility and fertilization as they pass through the epididymis (Bedford, 1975). Early investigators thought that sperm maturation depended on epididymal secretions (Von Ebner, 1888; Hammar, 1897; Benoit, 1926). Young, from his studies in the guinea pig, however, believed that maturation was dependent only on the age of the spermatozoa (Young, 1929a, 1929b, 1931). More recent studies in a variety of species suggest that the epididymal environment is essential for sperm maturation (Bedford, 1975; Hinrichsen and Blaquier, 1980; Orgebin-Crist and Jahad, 1979).

Sperm maturation also depends on the presence of androgens (Orgebin-Crist, et al, 1975; Orgebin-Crist and Jahad, 1978). Androgens may affect maturation by a direct action on spermatozoa or, more probably, through an indirect action on the epididymal epithelium. A change in androgen levels could lead to changes in the intraluminal microenvironment of the epididymis, which supports sperm maturation.

In the present study estradiol valerate was used to inhibit the production and action of testosterone. The elemental composition of the intraluminal fluids of the seminiferous tubules, rete testis, and epididymis was then determined using micropuncture techniques and electron-probe microan-

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alysis. The results provide basic information regarding hormonal regulation of the elemental microenvironment in the intraluminal fluids of the testis and epididymis.

Materials and Methods

Male Sprague-Dawley rats, weighing from 350 to 550 g, were allowed to acclimate to their new environment for at least 1 week after shipment. They were then divided into two groups. Each animal in the treated group (n = 14) received an intramuscular injection of 2.5 mg of estradiol valerate (Delestrogen, 20 mg/ml in castor oil and 20% benzyl benzoate; E. R. Squibb & Sons, Inc., Princeton, NJ) on days 1 and 4. Estradiol valerate has a prolonged estrogenic effect and is used for estrogen replacement in women and for the palliative treatment of advanced carcinoma of the prostate in men (Delestrogen, 1976; Murad and Gilman, 1975). Each animal in the sham-treated group (n = 7) received an intramuscular injection of 0.125 ml of vehicle only (castor oil and 20% benzyl benzoate) on days 1 and 4.

On day 13 the rats were anesthetized with an intraperitoneal injection of Inactin (sodium 5-ethyl-5-(1-methyl propyl)-2-thiobarbiturate, 100 mg/kg body weight). Through a midline abdominal incision, the efferent ducts between the right testis and epididymis were ligated. The testis and epididymis were placed in the scrotum, and the wound was closed. The left testis and epididymis were exposed through a scrotal incision, and moistened with water-equilibrated mineral oil, and samples were obtained by micropuncture (Howards et al, 1975) from the seminiferous tubules and two areas of the epididymis: caput and distal cauda epididymis. Four hours after ligation of the right efferent ducts, a sample was obtained from the distended rete testis. Serum samples were obtained last. The animals remained anesthetized during the entire 4-hour period.

The microsamples were immediately centrifuged, and the supernatants were frozen. The frozen samples were shipped for preparation and analysis to the National Biotechnology Resource in Electron Probe Microanalysis at Harvard Medical School, where the concentrations of seven elements (sodium, potassium, chloride, phosphorus, calcium, magnesium, and sulfur) were measured using electron-probe microanalysis (Lechene, 1970). The mean and standard error of the mean were calculated for each element in each sampling area. The data were analyzed using Student's t-test for unpaired data.

Results

Sodium

The sodium concentration almost doubled (106.1 ± 3.4 to 182.8 ± 16.9 mmol/1, $P < 0.01$) in the caput epididymidis following estrogen treatment but did not change in the other sampling areas (Table 1).

Potassium

The concentration of potassium was not altered by estrogen treatment.

Chloride

Like that of sodium, the chloride concentration rose (16.5 ± 2.2 to 79.3 ± 10.8 mmol/1, $P < 0.01$) in the caput epididymidis after estrogen treatment. The chloride concentration also rose (24.4 ± 1.7 to 54.9 ± 3.9 mmol/1, $P < 0.01$) in the distal cauda.

TABLE 1. Elemental Concentrations in the Intraluminal Fluids of the Rat Seminiferous Tubules (SNT), Rete Testis (Rete), Caput, and Distal Cauda Regions of the Epididymis and Serum: Effect of Treatment with Estradiol Valerate (mmol/l; mean ± SEM)

		SNT	Rete	Caput	Distal Cauda	Serum
Sodium	Sham	152.4 ± 4.7	150.3 ± 14.8	106.1 ± 3.4	73.6 ± 12.4	141.3 ± 9.1
	Estrogen	137.1 ± 8.4	155.4 ± 16.8	182.8 ± 16.9(*)	113.0 ± 13.3	130.6 ± 5.2
Potassium	Sham	44.0 ± 2.6	12.4 ± 2.1	20.9 ± 0.8	33.4 ± 2.4	5.77 ± 0.53
	Estrogen	48.8 ± 5.7	11.3 ± 0.5	32.9 ± 3.8	38.2 ± 2.2	5.55 ± 0.27
Chloride	Sham	140.8 ± 6.0	122.8 ± 5.0	16.5 ± 2.2	24.4 ± 1.7	95.9 ± 6.7
	Estrogen	128.4 ± 10.7	133.5 ± 3.3	79.3 ± 10.8(*)	54.9 ± 3.9(*)	107.7 ± 4.4
Phosphorus	Sham	12.5 ± 1.4	2.00 ± 0.30	63 .7 ± 1.6	85.3 ± 1.0	2.54 ± 0.61
	Estrogen	17.4 ± 2.3	0.67 ± 0.12(*)	47.8 ± 3.2(*)	88.6 ± 9.6	2.28 ± 0.11
Calcium	Sham	0.43 ± 0.14	0.66 ± 0.15	0.93 ± 0.09	0.03 ± 0.06	0.38 ± 0.05
	Estrogen	0.44 ± 0.07	1.55 ± 0.21(*)	0.50 ± 0.07(*)	0.39 ± 0.06	0.60 ± 0.10
Magnesium	Sham	0.93 ± 0.22	0.25 ± 0.08	2.21 ± 0.41	0.24 ± 0.11	0.24 ± 0.08
	Estrogen	1.18 ± 0.14	0.44 ± 0.15	0.76 ± 0.16(*)	0.63 ± 0.15	0.47 ± 0.05
Sulfur	Sham	7.70 ± 0.88	6.51 ± 1.81	18.4 ± 1.0	9.9 ± 0.7	3.38 ± 0.38
	Estrogen	7.15 ± 0.72	4.26 ± 1.35	10.8 ± 1.0(*)	15.8 ± 1.7	4.20 ± 1.01

* Values for sham- and estrogen-treated animals are significantly different ($P < 0.01$).

Phosphorus

The concentration of phosphorus fell in both rete testis fluid (2.00 ± 0.30 to 0.67 ± 0.12 mmol/1, $P < 0.01$) and caput epididymidal fluid (63.7 ± 1.6 to 47.8 ± 3.2 mmol/1, $P < 0.01$) following estrogen treatment.

Calcium

The calcium concentration rose in the rete testis (0.66 ± 0.15 to 1.55 ± 0.21 mmol/1, $P < 0.01$) but fell in the caput epididymidis (0.93 ± 0.09 to 0.50 ± 0.07 mmol/1, $P < 0.01$).

Magnesium

The magnesium concentration fell (2.21 ± 0.41 to 0.76 ± 0.16 mmol/1, $P < 0.01$) in caput epididymidal fluid after estrogen treatment.

Sulfur

Like those of phosphorus, calcium, and magnesium, the concentration of sulfur fell (18.4 ± 1.0 to 10.8 ± 1.0 mmol/1, $P < 0.01$) in the caput epididymidis.

Estrogen treatment did not change the concentration of any element in seminiferous tubular fluid or serum.

Discussion

Estrogens suppress the secretion of gonadotropins by the pituitary (Steinbeck et al. 1971), inhibit the action of gonadotropins on the testis (Tcholakian et al, 1978), and inhibit the effect of testosterone on the epididymis (Lubicz-Nawrocki, 1974). Since these mechanisms reduce the circulating levels of androgens and inhibit the effects of androgens on the testis and epididymis, estrogen treatment can be used to study the androgen dependence of the testis and epididymis.

However, estrogen receptors have been identified in epididymal tissue (Murphy et al, 1980). The highest concentrations are in the cauda epididymidis (Danzo and Eller, 1979). Although no physiologic role for these epididymal estrogen receptors has been found, it is possible that estradiol can directly affect epididymal function, especially that of the caudal region.

Treatment with estrogen or an antiandrogen interferes with spermatogenesis. Steinbeck et al (1971) found spermatogenic arrest in histologic sections of testes from rats treated with estradiol for three weeks. Flickinger and Loving (1976) concluded that in rats given cyproterone acetate, germ cells develop up to the early spermatid stage and then begin to degenerate and die.

In the present study estrogen treatment had no effect on the elemental composition of intraluminal seminiferous tubular fluid. Androgens may exert their effects on spermatogenesis only at the level of the germinal epithelium and not through regulation of the intraluminal fluid environment of the seminiferous tubule. Additionally, the germinal epithelium may not have the absorptive and secretory capacity to alter the composition of seminiferous tubular fluid.

Estrogen treatment altered the concentrations of two elements in rete testis fluid: phosphorus and calcium. The biologic significance of these changes is not known.

Sperm maturation is a complex process that involves the development of motility, fertilizing ability, and the capacity to produce viable embryos. Evidence from several investigators implies that the development of these characteristics by spermatozoa occurs in the proximal epididymis (Gaddum, 1968; Hinrichsen and Blaquier, 1980; Orgebin-Crist, 1967; Orgebin-Crist et al, 1975).

Furthermore, sperm maturation is an androgen-dependent event (Orgebin-Crist et al, 1975). The effects of androgens on sperm maturation may be exhibited through androgen-directed synthesis of new ribonucleic acid and protein by the epididymal epithelium rather than through a direct effect on spermatozoa (Orgebin-Crist and Jahad, 1978, 1979).

The present study provides further evidence that the epididymal epithelium modifies the composition of epididymal fluid and that this capability is under hormonal control. Treatment with estradiol valerate, either through a direct effect or an indirect, antiandrogenic effect, markedly altered the elemental composition of caput epididymidal fluid.

Estradiol valerate had little effect on the elemental composition of caudal fluid: the chloride concentration increased. This implies that cauda epididymal function is androgen-independent or estrogen-insensitive. However, previous investigators reported that caudal function is androgen-dependent. After castration in the rabbit the sodium concentration in caudal fluid increased, while the concentrations of potassium and phosphate exhibited variable changes (Jones and Glover, 1973).

Three weeks after castration the reabsorption of sodium and water and the secretion of potassium were abolished in the perfused rat cauda epididymidis (Wong and Yeung, 1978). Although the distal caudal sodium concentration appeared to increase in the present study, this was not statistically significant ($P = 0.04$). Methodologic and species differences may have led to the discrepancies between previous studies and the present study.

It is possible to speculate that androgens act on the epididymal epithelium, which then regulates the inorganic composition of epididymal fluid and provides the proper milieu for sperm maturation. Although the specific biologic actions of these elements (Na, K, Cl, P, Ca, Mg, S) on sperm maturation are not known, their roles in general cellular physiology are well known: Na and K—cellular transport; P—cellular energy metabolism and acid–base balance; Ca and Mg—enzyme cofactors and cellular motility; S—protein structure. It is likely that these elements have the same functions in spermatozoa.

Optimal inorganic ion concentrations in the epididymis may be an important requirement for the proper function of proteins and other organic molecules that are involved in sperm maturation and transport. Inorganic ions may be directly involved in sperm maturation. Whatever its role in sperm maturation, the elemental composition of caput epididymidal fluid, in which sperm maturation is initiated, is very sensitive to treatment with estradiol valerate.

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