Effect of Vasectomy on the Osmolarity of Hamster Testicular and Epididymal Intraluminal Fluid

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Intraluminal fluids from the hamster seminiferous tubules and cauda, corpus, and caput epididymidis were obtained *in vivo* using micropuncture techniques. The effect of vasectomy on the osmolarity of these fluids was studied two weeks and four months postvasectomy. There was an overall decrease in the osmolarity of reproductive fluids at two weeks postvasectomy that was not apparent at four months postvasectomy.

Key words: testis, epididymis, vasectomy, osmolarity, hamster.

The effect of vasectomy on normal testicular and epididymal function is an important research topic of current interest. Changes in testicular and epididymal integrity, function, and luminal fluid composition as a result of vasectomy may be pertinent to understanding the problem of persistent male infertility after technically successful vasovasostomy.

The present study was conducted to determine the effects of vasectomy on the osmolarities of hamster testicular and epididymal intraluminal fluids.

Methods

Adult, male golden hamsters (*Mesocricetus auratus*) weighing 100–150 g each (Lakeview Hamster Colony, Newfield, N.J.) were maintained in constant-temperature housing with a 14-hour light/10-hour darkness cycle.

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Vasectomy

Treatment animals were bilaterally vasectomized using standard techniques either two weeks or four months before experimental use. These animals were anesthetized with sodium pentobarbital (Nembutal®, Abbott Laboratories, Chicago, Illinois), administered intraperitoneally in a dose of 200 mg/kg body weight. Hamsters in the short-term (two-week) treatment group were vasectomized through small scrotal incisions. Vasectomy in the long-term (four-month) treatment group was performed through a laparotomy incision. The vas deferens was dissected free from the major blood vessels prior to removal of a 5-mm section of the vas between 3-0 silk ligatures. Two ligatures were placed both proximally and distally to ensure occlusion. The testes and epididymides were returned to the scrotum. Muscle and skin layers were closed separately with interrupted 4-0-chromic and 4-0-silk sutures, respectively. Testes and epididymides were palpated after surgery to ensure scrotal location.

Micropuncture

Control and treatment animals were anesthetized 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. The testes and epididymides were approached through a scrotal incision and prepared for micropuncture as previously described (Howards, Johnson, and Jessee, 1975b). Animals micropunctured four months postvasectomy and their control group were administered a continuous intravenous infusion of 0.154 M saline (1.5 ml/hr) to maintain proper fluid balance during the micropuncture operation. Intraluminal seminiferous tubule fluid and fluids from the caput, corpus, and cauda epididymides were obtained by micropuncture (Howards, Jessee, and Johnson, 1975a). Blood was collected from each control and treatment animal by cardiac puncture at the end of the sampling period. Duplicate or triplicate samples of each fluid were obtained from each animal. All samples were

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sandwiched between columns of sudan-black stained, water-equilibrated mineral oil in collection pipettes to prevent evaporation. Collected microsamples were centrifuged at 13,500 *g* for 15 minutes in a refrigerated (0 C) IEC centrifuge to obtain cellfree fluids. These fluid samples were stored under oil in sealed collection pipettes at 20 C until analysis. Storage for one to three days under these conditions did not alter osmolarity.

Osmolarity Determinations

Animals two weeks and four months postvasectomy were assigned separate control groups; fluids from each treatment group were analyzed along with fluids from their respective controls. Osmolarities were determined for duplicate or triplicate aliquots (depending on sample volume available) of each reproductive tract fluid and blood sera sample by measuring freezing point depression. These measurements were made with a nanoliter osmometer (Clifton Technical Physics, Hartford, New York) as described by Johnson and Howards (1976a) and were expressed as milliosmoles per liter of water.

Data Analysis

The mean ± SE fluid osmolarities were determined for blood plasma and intraluminal fluids from the seminiferous tubules and three regions of the epididymis from the two treatment groups and their respective control groups. Data on each fluid from the two-week and four-month postvasectomy groups were compared to data from the corresponding fluids of their respective control groups by two-way analysis of variance followed by the Duncan's multiple range test. (For purposes of statistical analysis, $P < 0.01$ was considered significant.)

Results

Analysis of variance showed that reproductive tract fluid osmolarities of hamsters two weeks after vasectomy were significantly lower overall than those of control hamsters; however, range tests could not detect treatment-induced differences in specific fluids (Fig. 1). Caput and corpus epididymidal fluids were significantly hyperosmolar compared to serum in both two-week treatment animals and their respective controls. Cauda fluid was not significantly different from serum in either group. Seminiferous tubule fluid had a higher osmolarity than serum in control animals but not in animals in the two-week postvasectomy group.

Both two-week treatment and control blood sera contained elevated milliosmolar concentrations (Fig. 1). This was attributed to fluid loss during the prolonged periods of micropuncture. Subsequently, the four-month postvasectomy animals and their control group were administered continuous intravenous infusion of physiologic saline, as noted in the micropuncture methods.

Data from hamsters sampled four months after vasectomy and their concurrent control group are shown in Fig. 2. Blood sera from both treatment and control groups had normal osmolarities (297.4 and 298.6 mOsm/l, respectively). The overall significant treatment effect on osmolarity found at two weeks postvasectomy was not evident at four

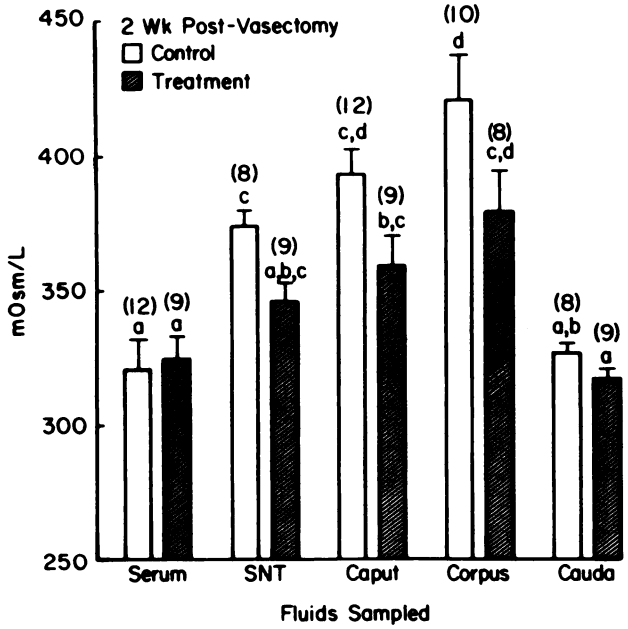

Fig. 1. Mean ± SE osmolarities of blood-serum and reproductive tract fluids from control hamsters and hamsters sampled two weeks after vasectomy. Mean ± SE bars sharing the same letter superscript are not significantly different ($P < 0.01$ was considered significant). The number of animals contributing to the mean value of each group is noted parenthetically above each bar. SNT = seminiferous tubule.

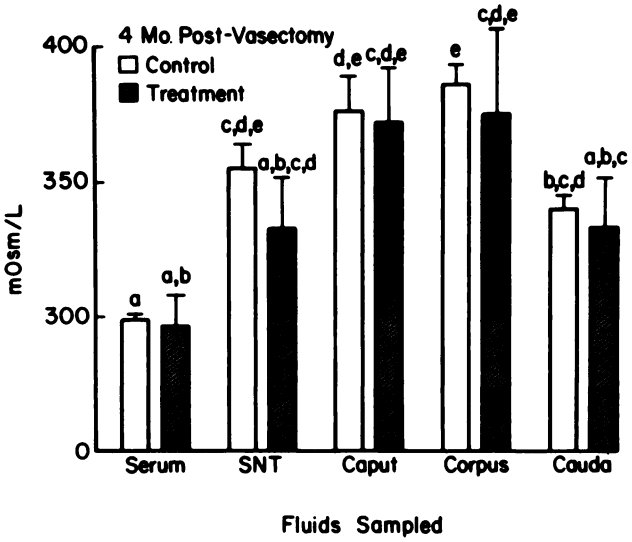

Fig. 2. Mean ± SE osmolarities of blood serum and reproductive tract fluids from control hamsters and hamsters sampled four months after vasectomy. Mean ± SE bars sharing the same letter superscript are not significantly different ($P < 0.01$ was considered significant). The number of animals contributing to each mean value was five in all cases. SNT = seminiferous tubule.

months postvasectomy. Neither did the long-term vasectomy induce any significant changes in the osmolarity of specific reproductive tract fluids. In this control group, all reproductive tract fluid osmolarities were significantly higher than serum osmolarity. Caput and corpus epididymidal fluids were significantly hyperosmolar to serum in the control group and the treatment group at four months postvasectomy.

Discussion

Epithelial secretions of the seminiferous tubules and the epididymal duct, together with the reabsorptive properties of the epididymal epithelium, provide the normal male's spermatozoa with an environment that is necessary for them to become fully mature. Vasectomy may cause some alterations, either transient or permanent, in male tract tubule function. Even permanent alterations in tubule physiology may be of no consequence unless vasectomized individuals wish to re-establish their fertility. Especially for these individuals, it is important for us to understand the effects of vasectomy on the male reproductive tract.

This article is concerned with the effects of vasectomy on osmolarity, a parameter that would reflect changes in ionic gradients, organic molecule concentrations, fluid movement, etc. Johnson and Howards (1976a) demonstrated the hyperosmolarity of seminiferous tubule, epididymal tubule, and vas deferens luminal fluids to blood serum in the normal hamster. Salisbury and Cragle (1956), Scott et al (1963), and Levine and Marsh (1971) also found reproductive fluids to be hyperosmolar to blood in the bull, ram, and rat, respectively. However, Tuck et al (1970) found seminiferous tubule fluid to be iso-osmolar to blood plasma in the rat, and studies in the ram, bull, boar, rat, hamster, and wallaby have found rete testis fluid to be iso-osmolar to blood plasma (Setchell, 1974; Tuck, 1970).

In the present study, intraluminal fluids from the seminiferous tubules, caput, and corpus epididymidis for both groups of control hamsters and cauda epididymidal plasma from one group of control hamsters were found to be hyperosmolar compared to blood serum. This is in agreement with a previous report by Johnson and Howards (1976a). Normal osmolarities increased to a maximum in the corpus and declined in the cauda to levels iso-osmolar or slightly hyperosmolar to blood serum. How this hyperosmolarity is maintained is not well understood. Active solute pumps (Setchell, 1970; Wong, 1976; Turner, Hartmann, and Howards, 1979) and secretion of organic molecules (Hansson, et al, 1976; Turner, Plesums, and Cabot, 1979) in the seminiferous tubule probably contribute to the hyperosmolarity of its fluids. The well-known removal of water from and secretion of such compounds as glycerylphosphorylcholine, carnitine, and sialic acids into the epididymal lumen are undoubtedly involved in the maintenance of epididymal intraluminal hyperosmolarity.

Short-term and long-term vasectomy do not significantly alter the osmolarity of the individual regions sampled; however, there was an overall

reduction in osmolarity for reproductive fluids for the entire treatment group at two weeks postvasectomy. There was no overall treatment effect evident at four months postvasectomy. These data imply that the testis and epididymis compensate for a transitory reduction in osmolarity within four months postvasectomy. Previous evidence for transitory changes in male tract physiology after vasectomy was presented by Johnson and Howards (1975).

Other investigations on the effects of vasectomy on the blood–testis and blood–epididymal barriers have demonstrated that changes in the transport characteristics of the hamster seminiferous tubule and epididymal epithelium were still present at four months after vasectomy (Turner, D'Addario, and Howards 1979). Vasectomy may result in damage to epithelial cells initially; however, four months after vasectomy, the epithelium may be substantially, but not completely, repaired. It is possible that evidence of residual malfunction can only be found by examining these subtle changes in the blood–testis and blood–epididymal barriers and/or in other aspects of epithelial cell function. Whether these alterations in normal tubule physiology ever return to normal is not known at present.

Ancillarily, testicular length was measured in a group of control group hamsters and in the four months postvasectomy group. Johnson and Howards (1975) previously reported a significant decrease in hamster testicular weight at the two weeks postvasectomy period. Because of protocol restrictions, we could not measure fresh testicular weights; however, as in the osmolarity data discussed previously, hamster testicular lengths (indicator of total size) were not significantly different (t test, $\alpha = 0.05$) from controls four months after vasectomy. Evidence from studies using the guinea pig also suggest that testicular size recovers from the initial effects of vasectomy (Johnson and Howards, 1976b).

The purpose of the present study was to investigate the effects of vasectomy on the osmolarity of testicular and epididymal intraluminal fluids. We have demonstrated a transient effect of vasectomy on reproductive tract fluid osmolarity at two weeks postvasectomy. Fluid osmolarity and testicular length were normal at four months postvasectomy. It is not known whether such transitory changes have an influence on testicular and epididymal function; however, it is known from the previous investigations already discussed that these transitory changes are accompanied by subtle alterations more permanent in nature.

References

Hansson V, Weddington SC, French FS, McLean W, Smith A, Nayfeh SN, Ritzen EM, Hagenas L. Secretion and role of androgen-binding proteins in the testis and epididymis. J Reprod Fertil [Suppl] 1976; 24:17–33.

Howards SS, Jessee S, Johnson A. Micropuncture and microanalytic studies of the effect of vasectomy on the rat testis and epididymis. Fertil Steril 1975a; 26:20–28.

Howards SS, Johnson A, Jessee S. Micropuncture and microanalytic studies of the rat testis and epididymis. Fertil Steril 1975b; 26:13–19.

Johnson A, Howards SS. Intratubular hydrostatic pressure in testis and epididymis before and after vasectomy. Am J Physiol 1975; 228:556–564.

Johnson A, Howards SS. Hyperosmolality in intraluminal fluids from hamster testis and epididymis: a micropuncture study. Science 1976a; 195:492–493.

Johnson A, Howards SS. Intratubular hydrostatic pressure in testis and epididymis before and after long-term vasectomy in the guinea pig. Biol Reprod 1976b; 14:371–376.

Levine N, Marsh DJ. Micropuncture studies of electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and vas deferens in rats. J Physiol 1971; 213:557–570.

Salisbury GW, Cragle RG. Freezing point depressions and mineral levels of fluids of the ruminant male reproductive tract. Proceedings of the Third International Congress on Animal Reproduction 1956; 1:25–28.

Scott TW, Wales RG, Wallace JC, White IG. Composition of ram epididymal and testicular fluid and the biosynthesis of glycerylphosphorylcholine by the rabbit epididymis. J Reprod Fertil 1963; 6:49–59.

Setchell BP. Testicular blood supply, lymphatic drainage, and secretion of fluid. In: Johnson AD, Gromes WR, Vandemark NL, eds. The Testis. Vol. 1. New York: Academic Press, 1970:197.

Setchell BP. Secretions of the testis and epididymis. J Reprod Fertil 1974; 37:165–177.

Tuck RR, Setchell BP, Waites GMH, Young IA. The composition of fluid collected by micropuncture and catheterization from the seminiferous and rete testis of rats. Pfluegers Arch 1970; 318:225–243.

Turner TT, D'Addario DA, Howards SS. Effects of vasectomy on the blood–testis barrier of the hamster. J Reprod Fertil 1979; 55:323–328.

Turner TT, Hartmann PK, Howards SS. Urea in the seminiferous tubule: evidence for active transport. Biol Reprod 1979; 20:511–515.

Turner TT, Plesums JL, Cabot CL. Luminal fluid proteins of the male rat reproductive tract. Biol Reprod 1979; 21:883–890.

Wong PYD. Uptake of sodium into rat isolated seminiferous tubules *in vitro*. Jpn J Physiol 1976; 26:321–331.