cence. If, for example, half of the cell fluorescence recorded before Triton treatment had been due to dye in vesicles, Triton should have increased the value by a factor of at least (0.5 × 1.19 + 0.5 × 30) ≃ 16. The number 1.19 arises from the *p*H correction described in (*22*).

24. When lymphocytes were incubated with vesicles of distearoyl lecithin (transition temperature, 56°C) containing 10 m*M* 6-CF (self-quenched by only a factor of 2), the cells developed clearly defined fluorescent rims, indicating dye in vesicles at the cell surface. Binding of vesicles to cell membranes has recently been described independently by L. Huang, K. Ozato, and R. E. Pagano [*Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 1703 (1976)] and R. Martin and R. C. MacDonald [*J. Cell Biol.* **70**, 515 (1976)].
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Hyperosmolality in Intraluminal Fluids from Hamster Testis and Epididymis: A Micropuncture Study

Abstract. *Intraluminal fluids from the hamster seminiferous tubules, rete testis, and caput, corpus, and cauda epididymidis were obtained in vivo by micropuncture. Rete testis fluid is isosmolar with serum. Fluids from the seminiferous tubules and all sites in the epididymis are hyperosmolar.*

Spermatozoa are neither motile nor fertile immediately after their release from the germinal epithelium. By the time they reach the cauda epididymidis, they have acquired the capacity for fertility and motility. Studies of sperm held by ligation in the proximal epididymis (*1, 2*) have shown that this maturation is not the result of aging alone, but is partially dependent on exposure to the epididymal milieu. It seems likely that maturation has some correlation with the progressive changes that occur in the ionic and organic constituents of the tubule fluids along the length of the excurrent ducts (*3*). However, knowledge of the composition of these fluids is limited. One basic factor which has received only minimal attention is their osmolality.

For osmolality studies in the boar (*4*), ram (*5, 6*), and hamster (*6*), testes and epididymides were obtained at slaughter, and the contents squeezed out from cut tubules. The development of a technique for cannulation of the rete testis of the conscious ram (*7*) marked the first time that fluid had been collected from the male reproductive tract in vivo. Later, micropuncture techniques made it possible to obtain fluid directly from the seminiferous tubules and proximal ductus epididymidis in vivo (*8, 9*). Here we report the results of the determination of osmolality in fluids obtained by micropuncture from the seminiferous tubules, rete testis, and five regions of the epididymis of the golden hamster, *Mesocricetus auratus*.

Adult male golden hamsters (100 to 170 g; Lakeview Hamster Colony) were anesthetized and prepared for micropuncture as described previously (*10, 11*). Samples of rete testis fluid were collected 2 to 3 hours after ligation of the efferent ducts. All samples were sandwiched between columns of water-equilibrated mineral oil in the collection pipettes to prevent evaporation, and were centrifuged at 13,460*g* for 15 minutes to separate the spermatozoa. Blood was collected from each animal by cardiac puncture at the end of the experiment.

Osmolality was determined on duplicate portions of each sample by measuring freezing-point depression with a nanoliter osmometer (Clifton Technical Physics). A special sample holder was constructed for the osmometer with sample wells that extend below the surface of the cooling module; the wells were thus in line with the cooling waves generated by the stage. To ensure proper thermal maintenance of all samples, a compound (Dow Corning 340 silicone) that would retard heat transfer was used as an interface between the sample holder and the cooling module. By means of a vertical transfer apparatus (Bunton Instrument) and constriction pipettes with 60 μm tips, 5- to 7-nl volumes were transferred to the osmometer from the samples, which were either sandwiched between columns of oil in the collection pipettes or deposited under oil in glass sample dishes. A standard curve was run with each group of three samples. Samples were either analyzed on the day of the experiment or stored under oil at −20°C and analyzed within 3 days. Storage for 1 to 3 days did not alter the osmolality.

The osmolalities in the seminiferous tubules (384.1 milliosmoles per kilogram) and all regions of the epididymis studied were significantly higher ($P < .01$) than the serum osmolality (Fig. 1). Rete testis fluid osmolality was not different from serum ($P < .05$). The osmolality decreased progressively down the length of the ductus epididymidis from 417.0 and 408.4 mosmole/kg in the caput and corpus, respectively, to 358.5 mosmole/kg in the proximal cauda ($P < .01$), then to 339.7 and 331.6 mosmole/kg in the distal cauda and epididymal vas, respectively ($P < .05$).

To determine whether the hyperosmolality of our samples was due to enzymatic degradation of the constituents of the fluids after they were collected, several proximal cauda samples were centrifuged in a cold room immediately after collection and held on ice until their transfer to the osmometer. The analysis was completed within 3 hours after collection. No significant difference was observed between the value (± standard error) of 340.4 ± 9.0 mosmole/kg ($n = 7$) for the cold samples and the original value of 358.5 ± 7.4 mosmole/kg ($n = 21$) for samples centrifuged and held at room temperature.

Additional experiments were performed to determine whether the samples were losing water into the surrounding oil. A small volume of tritiated water was injected into a segment of the ductus epididymidis in the caput and cauda. Then, a sample was withdrawn from the same location by micropuncture, centrifuged, and either left in the collection pipette or deposited under oil in a sample dish. In both cases, duplicate portions of the tubule fluid and of the oil near the fluid-oil interface were transferred to glass vials for liquid scintillation counting. No significant difference from background was ever observed for any of the oil samples, while there were as many as 20,000 count/min in a comparable portion of tubule fluid.

Hyperosmolality in the fluids of the male reproductive tract has been mentioned but not emphasized or statistically documented in previous literature. Several investigators reported that the fluids they collected from the testis and epididymis of large slaughtered animals were hyperosmolar, although the samples may have been contaminated or have undergone postmortem changes. Salisbury and Cragle (*12*) found that the osmolalities in the testis and cauda epididymidis of the bull tended to be higher than those in both blood and semen. Similarly, Scott *et al.* (*5*) reported that the osmolalities in the testis, caput and cauda epididymidis, and vas deferens of the ram were hyper-

osmolar to 0.9 percent NaCl, although they did not compare their results with the plasma osmolality.

Two previous reports of the osmolality of fluids obtained by micropuncture from the male reproductive tract are in conflict. Tuck *et al.* (*8*) reported no significant difference between seminiferous tubule fluid and plasma in the rat. In contrast, Levine and Marsh (*9*) found that rat seminiferous tubule fluid was significantly hyperosmolar when compared with plasma. The latter investigators also presented in tabular form the results of their studies of the osmolality in the epididymis and vas deferens, but did not discuss them anywhere in the text of their report. Our analysis of their data reveals that only the fluid in the caput epididymidis is isosmolar with plasma. The fluids from all other areas studied are significantly hyperosmolar.

In contrast to these findings of hyperosmolality, all studies of the osmolality of rete testis fluid to date (in the ram, bull, boar, rat, hamster, and wallaby) have shown that it is isosmolar with plasma (*13*).

In our experiments with the hamster, the extent of the hyperosmolality, particularly in the seminiferous tubules and caput and corpus epididymidis, is greater than that recorded for other species and may be due to species differences. It is interesting that, despite differences in collection technique, our value of 339.7 ± 3.4 mosmole/kg for the cauda epididymidis of the hamster is not different from the value of 323 ± 27.4 mosmole/kg reported by Jones (*6*).

It is not clear which constituents of the testicular and epididymal luminal fluids are responsible for such high osmolalities. The tendency for water to flow across a barrier in vivo does not always correlate directly with differences in total solute concentration across that barrier. Other factors such as the reflection coefficients of the solute molecules and the permeability of the barrier to water must be considered. We have shown that the half times for the entry of water into the seminiferous tubules and cauda epididymidis are 18.0 ± 13.2 and 24.0 ± 13.4 minutes, respectively (*14*). Thus, the permeability to water is finite, although true permeability constants cannot be calculated until the area-to-volume relationships of the tubules are determined. However, even if the physiologically operative pressures were somewhat lower than the pressure measured by the freezing-point depression method, evidence is accumulating that it may be maintained primarily by the organic constituents of the fluids, rather than by the inorganic ions. Levine and Marsh (*9*) documented in the rat a significant discrepancy, increasing down the length of the epididymis, between the measured osmolality and the osmolality that could be calculated from the ionic composition. Our measurements of the sodium and potassium ion concentrations in the five regions of the hamster epididymis (*11*) have led to a similar conclusion. Sodium, potassium, and their accompanying anions account for approximately 135 mosmole of the 417 mosmole/kg measured in the caput and 60 mosmole of the 340 mosmole/kg measured in the distal cauda. Information concerning the nature of quantities of the organic constituents found in these fluids substantiates this theory. Brooks *et al.* (*15*) have shown that, in the rat, glycerylphosphorylcholine (GPC) is added to the epididymal plasma in the caput, while carnitine appears in the cauda. The concentrations of GPC (41 m*M*) and carnitine (63 m*M*) they observed in the cauda could account for approximately 30 percent of the missing osmolality.

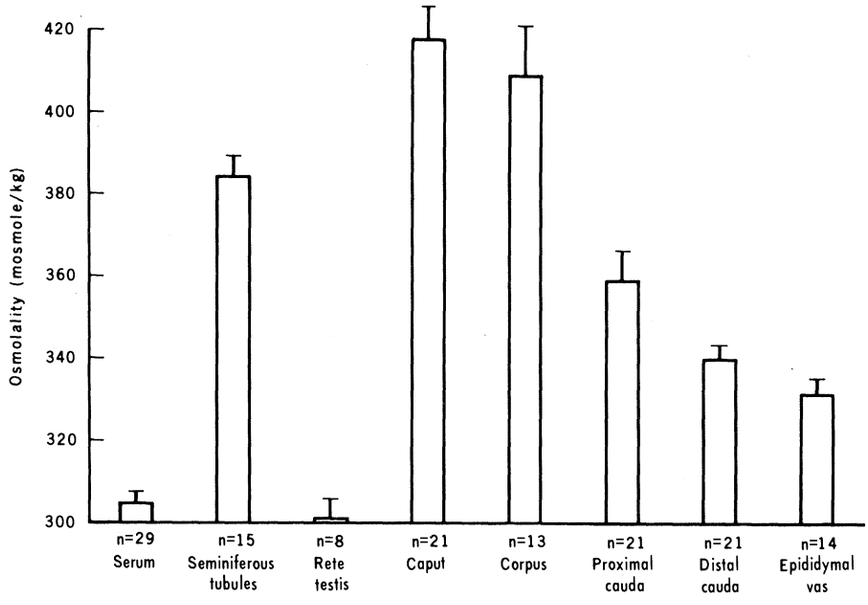

Fig. 1. Osmolality in the seminiferous tubules, rete testis, and five regions of the epididymis of the golden hamster. Each bar represents the mean ± standard error.

It is tempting to try to correlate the decrease in osmolality from the proximal to the distal epididymis with the increasing capacity of the spermatozoa for fertility and motility. It has been observed that sperm held in the lower corpus epididymidis of the hamster by ligation for 3 to 5 days are still incapable of fertilization, although they do show some improvement in motility (*1*). Perhaps, before they can develop fertilizing capacity, they must be exposed to the caudal fluid, which has a lower osmolality than that in the corpus. Before this hypothesis can be confirmed, further work must be done to demonstrate the extent to which the sperm are dependent for maturation on the composition of the epididymal plasma in general, and its osmolality in particular.

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