Concentrations of *myo*-inositol in the luminal fluid of the mammalian testis and epididymis

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Summary. The concentration of free *myo*-inositol was measured in the luminal fluid removed by micropuncture from the testis and epididymis of rat, hamster, rabbit, rhesus monkey, baboon, ram and boar. The highest concentration (mM) was found in the epididymal luminal fluid from the cauda epididymidis of the hamster (90) and the rat (50), and the lowest was seen in the rabbit (<0.5), and boar (1.0–1.2), whilst the rhesus monkey (17), the baboon (1.3) and the ram (0.9–5.9) were intermediate. The species also differed with respect to the changes in luminal inositol concentration along the epididymis.

Introduction

The presence of *myo*-inositol in the secretions of the male reproductive tract of different species has been known for many years (Mann, 1951, 1954; Hartree, 1957; Setchell, Dawson & White, 1968). *Myo*-inositol can be synthesized from glucose in the rat testis (Eisenberg, 1967) and Middleton & Setchell (1972) confirmed that the inositol present in the testis of the ram is almost exclusively derived from blood glucose rather than blood inositol. There appears to be little uptake of inositol into the testis or epididymis (Middleton & Setchell, 1972; Lewin, Yannai, Sulimovici & Kraicer, 1976), probably reflecting the ability of the blood–testis barrier to exclude a molecule of this type (see Setchell & Waites, 1975).

To our knowledge there have been no studies showing the precise distribution of *myo*-inositol in the luminal fluid of the testis and along the epididymis in different species. An earlier study (Hinton, Setchell & White, 1976) showed that it was possible to measure the concentration of free *myo*-inositol in the luminal fluid of the rat testis and epididymis; a concentration of approximately 50 mM was reached in the luminal fluid of the ductus deferens. We have now carried out more detailed studies of the distribution of free *myo*-inositol in the testicular and epididymal luminal fluid of different mammalian species.

Materials and Methods

Animals

Adult male animals were used unless otherwise stated. Porton-Wistar rats weighing 350–450 g were obtained from the Institute colony and golden hamsters, 120–160 g, were purchased from Wrights Ltd, Chelmsford, U.K. Rabbits (New Zealand White), 3–5 kg, were obtained from Morton Commercial Rabbits, Stansted, U.K., and 2 rams (Clun Forest), 50–70 kg, and 1 boar (Large White), 300 kg, were from the Institute's experimental farm stock. The collection of micropuncture samples from the rhesus monkeys (*Macaca mulatta*) and from a young baboon (5 years old: *Papio cynocephalus*) were performed at the Department of Anatomy, University of Birmingham, with the help of Dr John Marston.

Micropuncture

Testicular and epididymal luminal contents were collected from each species as previously described (Hinton, Dott & Setchell, 1979a; Hinton, Snoswell & Setchell, 1979b) and centrifuged to remove the spermatozoa (Hinton *et al.*, 1976); the supernatants were stored at -20°C .

Inositol assay

We used an established yeast bioassay as described by White & Black (1975) modified for the small micropuncture samples (Hinton *et al.*, 1976). The yeast *Kloeckera apiculata* has a specific growth requirement for inositol and thiamine; the assay has been successfully used to measure inositol in the secretions of the male reproductive tract (Hartree, 1957; Setchell *et al.*, 1968). Samples, 2–20 nl, were injected into a droplet of glass-distilled water positioned at the bottom of a siliconized glass test-tube (0.65 mm diam., 50 mm long: G.W.S., London, U.K.). The tubes were placed in an oven at 80°C for 20 min to dry the sample, and then capped and placed in another oven at 120°C for 2.5 h to be sterilized. Sterile assay medium was prepared by the method of White & Black (1975) except that only a few drops of sodium hydroxide were needed to bring the final assay medium to pH 5.0 and not 2.5 ml as originally stated.

Several loopfuls of yeast which had been subcultured for 3 days on agar slopes were transferred into a bottle containing 10 ml sterile distilled water until there were approximately 20×10^6 yeast cells/ml. A 100 μl aliquot of this yeast suspension was then transferred into a second bottle containing sterile distilled water, and 10 ml sterile assay medium were then added. Care was taken at all times to prevent contamination of the sterile solutions. The final yeast suspension was taken up into a sterile 10 ml glass syringe which was designed to fit an Arnold Hand Microapplicator (Burkard, Rickmansworth, U.K.) and 50 μl of the suspension were placed at the bottom of each glass tube containing the sterile dried sample. The glass tubes were then sealed using a gas-oxygen flame. The samples in the tubes were thoroughly mixed and the tubes kept in an incubator at 25°C for 3 days to allow growth of the yeast. The samples were thoroughly mixed on each day during the incubation period. After 3 days a 10 μl sample was removed from each tube and the number of yeast cells in the sample was counted in a haemocytometer chamber. Inositol standards (2–64 ng) were prepared with each assay and samples and standards were assayed in duplicate. A linear response was always obtained over the concentration of standards used.

Table 1. Mean $\pm$ s.e.m. concentrations (mM) of free *myo*-inositol in the luminal fluid of the testis and epididymis (see Hinton *et al.*, 1979a, for the sites of puncture) of animals of various species

	Rat	Hamster	Rhesus monkey	Baboon	Boar	Ram	Rabbit
No. of animals	4–8	4–6	3	1	1	2	3
Seminiferous tubule fluid	1.83 ± 0.15	3.18 ± 0.25	9.45 ± 1.56	—*	—*	—*	2.10
Rete testis fluid	2.49 ± 0.12	3.06 ± 0.62	—*	—*	5.60	7.6, 3.7	—*
Initial segment (Site 1)	20.97 ± 3.5	—*	—	—*	—*	—*	—*
Proximal caput (Site 2)	8.68 ± 0.88	49.22 ± 6.23	25.11	3.50	—*	9.3, 9.3	35.4
Distal caput (Site 3)	6.85 ± 0.44	43.76 ± 5.29	—*	—*	—*	—*	—*
Mid corpus (Site 4)	5.02 ± 0.46	43.06 ± 6.16	18.26 ± 1.10	5.90	—*	6.00, 6.2	—*
Distal corpus (Site 5)	5.58 ± 0.21	47.37 ± 4.19	—*	—*	—*	—*	1.44
Proximal cauda (Site 6)	29.47 ± 1.60	58.67 ± 6.31	17.02 ± 0.56	1.30	1.22	5.10	<0.5
Distal cauda (Site 7)	31.39 ± 0.28	90.22 ± 9.98	17.40 ± 2.38	—*	1.00	5.40, 0.94	<0.5
Ductus deferens (Site 8)	49.17 ± 2.73	83.11 ± 6.27	—*	—*	1.00	5.90, 2.3	<0.5
Plasma	0.082 ± 0.01	0.085 ± 0.01	—*	—*	—*	—*	—*

* Not tested.

Results

As shown in Table 1, the highest concentration of *myo*-inositol was found in the luminal fluid of the hamster (90 mm) and rat (50 mm) epididymis. In the hamster, *myo*-inositol increased approximately 15-fold in the luminal fluid from the testis to ductus deferens; a slight decline was seen from distal caput to mid corpus (sites 3 and 4; see Hinton *et al.*, 1979a). In the rat, however, there was an initial increase in the concentration of *myo*-inositol in the luminal fluid of the initial segment; the concentration declined towards the corpus but then increased in the cauda epididymidis region to reach approximately 40 mM. There appeared to be a slight decline in concentration of *myo*-inositol in the luminal fluid along the monkey epididymis and little change in the young baboon, but values in the ram, boar and rabbit epididymis fell sharply.

Considerable amounts of fluid are reabsorbed by the epididymis, particularly the caput, leading to a progressive increase in spermatocrit, in the bull and boar (Crabo, 1965) and rat (Levine & Marsh, 1971). From the spermatocrit values in Table 2, it is possible to calculate what the inositol concentration would be if no inositol were absorbed with this fluid (Table 2).

Table 2. Observed (mean $\pm$ s.e.m. for 7–13 observations for spermatocrit and 4–8 for inositol concentrations) and calculated mean values (mM) of inositol concentration in the luminal fluid of the rat epididymis (see Hinton *et al.*, 1979a, for the sites of puncture)

	Rete testis fluid	Epididymis						
		Proximal caput (Site 2)	Distal caput (Site 3)	Mid corpus (Site 4)	Distal corpus (Site 5)	Proximal cauda (Site 6)	Distal cauda (Site 7)	Ductus deferens (Site 8)
Spermatocrit % (S)	3.3 $\pm$ 0.25	20.6 $\pm$ 1.11	28.9 $\pm$ 1.22	35.6 $\pm$ 1.29	39.9 $\pm$ 2.14	45.2 $\pm$ 2.4	55.5 $\pm$ 1.73	59.2 $\pm$ 1.77
Vol. fluid/vol. sperm. (F) (F = (100 - S)/S)	29.3	3.85	2.46	1.81	1.50	1.21	0.80	0.68
Observed conc. [1]	2.49 $\pm$ 0.12	8.68 $\pm$ 0.88	6.85 $\pm$ 0.44	5.02 $\pm$ 0.46	5.58 $\pm$ 0.21	29.47 $\pm$ 1.6	31.39 $\pm$ 0.28	49.17 $\pm$ 2.73
Calculated conc. (i)* (ii)†	—	18.95	29.66	40.31	48.64	60.30	91.21	107.30
	—	—	—	—	6.06	7.50	11.36	13.36

* From $RTF = ([1_{RTF}] \times F_{RTF})/F_{Sites\ 2-8}$

† From mid-corpus = $([1_{Site\ 4}] \times F_{Site\ 4})/F_{Sites\ 5-8}$

Discussion

These results clearly show that there are species differences in the concentration of inositol in the testicular and epididymal luminal fluid. It seems likely that the inositol found in the luminal fluid of the rat epididymis from caput to distal corpus is at least partly of testicular origin since the testis is capable of synthesizing inositol from blood glucose (rat: Eisenburg, 1967; ram: Middleton & Setchell, 1972) and the initial increase seen in the caput could be due to water re-absorption at this point (Table 2). However, the increase in the concentration of *myo*-inositol in the luminal fluid of the cauda epididymidis is probably not of testicular origin and its presence cannot be attributed to a concentration effect (Table 2). If the observed concentration had been less than that calculated, then some inositol must have been lost by utilization or absorption, and there would be no need to postulate further secretion to explain the rise in concentration. As the observed concentration was greater than that calculated, then the inositol must have been added to the luminal fluid by synthesis or concentration from the blood by the epididymal cells.

Inositol is probably synthesized by the epididymis because Robinson & Fritz (1979) have shown that the rat epididymis contains the necessary enzymes for the biosynthesis of inositol

from glucose; the activities were sufficient to account for the high levels of free *myo*-inositol seen in the luminal fluid of the cauda epididymidis. Evidence against the accumulation of inositol by the epididymis comes from the work of Lewin *et al.* (1976) who found that injected radioactive *myo*-inositol was not present in any appreciable levels in the rat epididymis.

Between the caput and corpus epididymidis of the rat, spermatozoa develop the potential for directional motility (Hinton *et al.*, 1979a) and probably undergo several changes in their membranes (Dacheux, 1977). Metabolic changes in spermatozoa during maturation (Voglmayr, 1975) may be associated with the decrease in inositol concentrations in the luminal fluid between the caput and corpus. In the ram, boar and rabbit, a similar difference in inositol concentration probably occurs between the caput and corpus, but unlike the changes in rats and hamsters, the decrease was continued in the cauda. This decrease has already been observed in ram and rabbit epididymal tissue by Voglmayr & Amann (1973) and Voglmayr *et al.* (1977). The inositol is probably utilized by the spermatozoa or the epithelium of the cauda epididymidis, and could be involved in the suppression of motility of the spermatozoa or their energy metabolism whilst they are stored in the cauda epididymidis (Glover & Nicander, 1971).

It is not clear why different species have different concentrations of inositol in the testicular and epididymal luminal fluid, but similar differences have been observed for carnitine (Hinton *et al.*, 1979b) and for glycerophosphocholine (Hinton & Setchell, 1980). For the rat, inositol, carnitine, glycerophosphocholine, phosphocholine and inorganic phosphate contribute about 200 mosmol/kg water of the final osmolarity of the luminal fluid in the cauda epididymidis (330 mosmol/kg water; Levine & Marsh, 1971). The inorganic ions, sodium, potassium, chloride and bicarbonate, contribute about 100 mosmol, and the remaining 30 mosmol/kg water is probably due to lactic acid, sialic acids and some amino acids such as glutamate and glutamine.

We thank Professor I. B. Fritz for helpful discussions. The work was financially supported by the World Health Organization.

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Received 12 June 1979