the depletion of tissue DBH content and this depletion can be maintained while the stimulus is continued[2,10], and third, the half-life of the enzyme in plasma is now estimated to be about 7 h, using homologous rat DBH[11]. Accordingly, it can be suggested that fasting for 48 h depletes the DBH content in adrenal medulla and spleen. Released DBH should increase in plasma after the stimulus for a few hours, and later clear; because of the persistence of the stimulus (48 h) and the maintained depletion, plasma DBH levels decrease below the basal value.

After 48 h of cold exposure, the adrenal DBH content is significantly increased; on the contrary, the splenic DBH content is significantly decreased (table). These results suggest, on the one hand, that the adrenal activity is diminished with increase of its DBH content as a result of an impaired release, and on the other hand, that splenic stimulation gives rise to depletion of DBH, although this is less than is seen in the fasting group (table). These results agree with previous reports showing preferential sympathetic nervous system activation (increased plasmatic norepinephrine), whereas the adrenal medulla is only marginally affected after cold exposure[12]. Plasma DBH is also significantly decreased, but less than in the fasting group (table), which is logical since spleen depletion in the cold is smaller than in fasting and, moreover, adrenal exocytosis seems to be practically nonexistent.

Finally, when both stimuli are simultaneously employed, DBH activity is significantly decreased both in adrenal gland and spleen. Plasma glucose and plasma DBH activity are decreased too, both significantly when compared to controls and in a similar way to that seen in the fasting group (table), which suggests, in this situation, the preponderance of the effect of fasting on the sympathoadrenal response.

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Biochemical changes in some acrosomal enzymes of spermatozoa during maturation

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Summary. Goat spermatozoal hyaluronidase and acrosin show significantly increased activities during transition from caput to cauda epididymis. The activity of alkaline phosphatase decreases during spermatozoal transport through epididymis.
Key words. Goat, male reproductive organs; epididymis, caput; epididymis, cauda; testicular fluid; spermatozoa, maturation; enzymes, acrosomal.

Mammalian spermatozoa acquire their motility and fertilizing ability during their transport through the epididymal tract and in the presence of an epididymal fluid environment of special composition[2]. In this process of maturation, changes of spermatozoal motility[3], morphology[4], membrane properties[5], and of acrosomal enzymes, namely phosphatase[6], proacrosin[7] and surface ATPase[8], and other spermatozoal constituents[9] occur during the transition through epididymis. However, almost nothing is known about the biochemical changes that occur in the content of hydrolytic enzymes present in the sperm acrosome during epididymal maturation. In the present investigation, changes in the activities of acrosomal enzymes namely phosphatases, hyaluronidase and acrosin, during maturation of spermatozoa in different regions of epididymis, were demonstrated.

Materials and methods. Male reproductive organs of sexually mature goats were collected from the slaughter house immediately after slaughtering and were brought to the laboratory. The cauda and caput portions of the epididymis were carefully removed and were minced by a fine scalpel[10]. The rete testicular fluid, containing spermatozoa, was collected following the method of Voglmayr et al.[11]. The sperm suspensions (in saline) were centrifuged at 6000 × g for 15 min at 4 °C. The resultant sperm pellets were washed thrice with saline and resuspended in 0.5% Triton ×100 for 30 min at 37 °C[12]. After extraction, the supernatants were collected by centrifugation of the sperm suspension at 10,000 × g for 10 min and were used to assay the phosphatases and hyaluronidase. Acid and alkaline phosphatase activities were determined according to the method of Michell et al.[13] using para-nitrophenyl phosphate (Merck, Germany) as substrate. Hyaluronidase activity was determined following the method of Zaneveld et al.[14] using hyaluronic acid (Sigma) as substrate.

For assay of acrosin, spermatozoa were extracted with 10% glycerol-HCl, pH 2.8, containing 50 mM benzamidine (Sigma) for estimation of total activity. After centrifugation at 27,000 × g for 30 min at 4 °C, extracted supernatant was dialyzed (using Spectrapor 1) against 0.001 M HCl, pH 3.0 to

Changes in the activities of acrosomal enzymes of goat spermatozoa during epididymal transit

Spermatozoa collected from	Acid phosphatase (μg p-nitrophenol/h/ 10^7 sperm) n = 22	Alkaline phosphatase (μg p-nitrophenol/h/ 10^7 sperm) n = 22	Hyaluronidase (μg N-acetylglucosamine/ h/10^7 sperm) n = 16	Acrosin (mIU/min/10^7 sperm) n = 8
Rete testis (Rt)	17.6 ± 4.1	39.2 ± 1.9	6.9 ± 1.6	22.7 ± 2.7
Caput epididymis (Cp)	26.3 ± 8.9	168.3 ± 9.1	10.6 ± 4.0	39.6 ± 3.1
Cauda epididymis (Cd)	20.2 ± 2.4	14.2 ± 3.1	19.4 ± 1.8	50.7 ± 1.2
Level of significance				
Rt vs Cp	NS	$p < 0.001$	NS	$p < 0.005$
Cp vs Cd	NS	$p < 0.001$	$p < 0.05$	$p < 0.005$
Rt vs Cd	NS	$p < 0.001$	$p < 0.001$	$p < 0.001$

Each value represents mean ± SE. NS, Nonsignificant; n, number of observations.

remove benzamidine[15,16]. Acrosin activity was measured spectrophotometrically using N-α-benzoyl-L-arginine ethyl ester (Sigma) as substrate[17].

The data were subjected to statistical analyses according to Fisher and Yates[18].

Results. The table represents the changes in the activities of several enzymes of goat spermatozoa during their transit through the epididymis. Although the acid phosphatase activity increases in caput epididymal spermatozoa compared with that in rete testicular spermatozoa and the activity decreases in cauda epididymal spermatozoa compared with caput epididymal spermatozoa, the changes are not significant. A significantly increased alkaline phosphatase activity is found in caput epididymal spermatozoa over that in rete testicular spermatozoa, and the enzyme activity diminishes significantly from the caput to the cauda epididymal region. An increased hyaluronidase activity is found in caput epididymal spermatozoa in comparison to rete testicular ones, but the change is not significant. A significant increase in the activity of hyaluronidase occurs in spermatozoa during transit through the caput to the cauda epididymis. Total acrosin demonstrates a significant increase in activity when the spermatozoa travel from the rete testis to the caput epididymis, and from the caput to the cauda epididymis.

Discussion. The acquisition of fertilizing ability in mammalian spermatozoa occurs only after complete epididymal maturation, but its cause is still obscure. In contrast to acid phosphatase acitivity, significant changes in the activities of alkaline phosphatase of goat spermatozoa have been observed during their transport through the epididymis. More or less similar observations were reported by Terner et al.[6], who pointed out a significant decrease in alkaline phosphatase activity during the maturation process of rat spermatozoa. The cytoplasmic droplets, which contain several hydrolytic enzymes[19], are larger and more numerous in the caput epididymal spermatozoa. This may account for very high activity of alkaline phosphatase in the caput epididymis. The droplets also diminish in size during spermatozoal passage through the epididymis[4]. The lowering of the activity of alkaline phosphatase in spermatozoa during their transport from caput to cauda epididymis may be due to loss of cytoplasmic droplets. The epididymal fluids, the composition of which also varies from one region to another, may also play an important role in controlling the activities of acrosomal enzymes of spermatozoa undergoing maturation[10].

The increase in the activity of hyaluronidase, which helps in dissolving the cumulus oophorus surrounding the ovum during fertilization[20], of goat spermatozoa during their transport through the epididymis is of obvious significance. The reason for the decreased activity of acrosin in rete testicular and caput epididymal spermatozoa may be due to the presence of proteinase inhibitors[21]. These naturally-occurring inhibitors remain associated with the enzyme[22,23]. The occurrence of increased acrosin activity in spermatozoa from cauda epididymis is of physiological significance, since the enzyme is needed and is known to be released from acrosomes[16] to facilitate the penetration of spermatozoa through the zona pellucida of the ovum[24].

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