POLYAMINES IN BOVINE EPIDIDYMAL SPERMATOZOA

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The stimulation of biosynthesis of putrescine and the polyamines spermidine and spermine has been observed in many processes of cell proliferation and differentiation in eukaryotic organisms (Tabor & Tabor, 1964; Williams-Ashman & Reddi, 1971; Cohen, 1971; Williams-Ashman *et al.*, 1972; Bachrach, 1973), although the functional significance of these observations is still unclear and has evoked much discussion (Bachrach, 1973; Russell, 1973). The enhancement of putrescine and polyamine production has been correlated with protein-, RNA- and DNA-synthesis (Russell, 1973), and these diamines are probably involved in the regulation of mitosis (Fillingame & Morris, 1973). Since an influence of polyamines on membrane functions has been shown (Tabor & Tabor, 1964; Lockwood & East, 1974), we have investigated the alterations of the polyamine content of epididymal spermatozoa during their maturation in the epididymis.

The epididymis of mammals is involved in two functions, namely that of maturation and storage of spermatozoa (Mann, 1964; Orgebin-Crist, 1969; Glover & Nicander, 1971). A great many biochemical processes have been examined in maturing spermatozoa, but have not included the metabolism of biologically active polyamines. The present study involved analysis of the content of putrescine, spermidine, spermine and histamine in spermatozoa taken from distinct regions of the bovine epididymis. In addition, the activity of ornithine decarboxylase (EC 4.1.1.17) and diamino oxidase (EC 1.4.3.6), two enzymes associated with polyamine metabolism, was studied in these spermatozoa.

Immediately after slaughter the epididymides of six bulls were cooled on ice and transported to the laboratory within 30 min. Each epididymis was divided into four segments: the cauda (Segment 4), the corpus (Segment 3) and the two parts of the caput epididymidis on either side of the flexure (Segments 2 (distal) and 1 (proximal)). These portions were then cut open with a razor blade and the spermatozoa from two epididymides removed by washing with Ringer's solution (Mann, 1964). The suspensions of spermatozoa thus obtained were centrifuged at 380 *g* at room temperature and the pellet resuspended in 5 ml Ringer's solution. Cell counts were performed on these suspensions. Microscopic examination of stained smears from the sperm suspensions showed no contamination with epithelial cells.

For the analysis of the polyamines the sperm suspensions were again sedimented by centrifugation, this time at 1300 *g*, and the pellet extracted with 5 ml of a 3% sulphosalicylic acid solution in 0.05 N-HCl. After homogenization at 0°C by ultrasonic treatment (28 times for 15 sec) the material was stored at -20°C for 16 hr. After thawing it was then centrifuged at 1500 *g* for 2 min and the polyamines determined in the supernatants according to the method of Marton *et al.* (1973) using column chromatography and an adapted automatic amino acid analyser.

For the estimation of enzymatic activity the extracts obtained from suspensions of spermatozoa after homogenization and centrifugation at 120,000 *g* were incubated with the proper substrates as follows: ornithine decarboxylase was determined by the method of Jones *et al.* (1972) using an incubation medium containing 0.75 mM-sodium potassium phosphate buffer, pH 6.8, 2 mM-EDTA, 0.5 mM-pyridoxalphosphate, and 5 mM-dithiothreitol. Diamino oxidase was determined by the method of Kusche *et al.* (1973) using 0.2 M-sodium potassium phosphate buffer, pH 7.6, as the incubation medium. Radioactivity was measured in a liquid scintillation spectrometer (Packard).

Table 1. The amine content (nmol/10⁸ cells) of the spermatozoa from the bovine epididymis

Amine	Segment 1	Segment 2	Segment 3	Segment 4
Putrescine	111.7 ± 72.4 (6)	8.5 ± 4.2 (5)	7.0 ± 2.1 (5)	2.5 ± 2.1 (6)
Spermidine	38.4 ± 27.2 (5)	4.5 ± 2.3 (5)	9.0 ± 4.7 (5)	1.2 ± 0.6 (5)
Spermine	58.2 ± 40.8 (4)	11.2 ± 8.8 (4)	25.4 ± 11.6 (3)	7.7 ± 6.8 (5)
Histamine	70.5 ± 63.2 (5)	1.6 ± 1.2 (3)	7.9 ± 0.6 (4)	1.9 ± 0.1 (2)

Values are Means ± S.D. with the number of determinations in parentheses.

TEXT-FIG. 1. The relative decrease in the amine content of bovine epididymal spermatozoa. See text for distinction of Segments.

The results of the amine determinations are shown in Table 1. During passage of spermatozoa from the caput to the cauda epididymidis the content of putrescine, spermidine, spermine and histamine decreased markedly. The percentage change in the sperm content of the four amines between Segments 1 and 4 is shown in Text-fig. 1. In Segment 3 the content of spermidine, spermine and histamine (but not that of putrescine) was higher than in the two adjoining segments. Diamino oxidase activity was detected in extracts of spermatozoa from all four segments, but the activity recorded was consistently less than 1.4 $\mu\text{g}/\text{mg}$ protein, i.e. too low to enable quantitative comparison between the four segments. Ornithine decarboxylase could not be detected in the spermatozoa.

These variations in polyamine content may influence the membranes of epididymal spermatozoa during their maturation, resulting in alterations of function. We hope to extend our observations concerning the polyamines and the two enzymes to testicular and ejaculated spermatozoa.

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