BRIEF COMMUNICATION

AMINO ACIDS, AMINES AND PEPTIDES OF RAM
EPIDIDYMAL SEMEN*

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Epididymal semen was obtained from rams with a fistula present in the vas deferens (Tadmor & Schindler, 1966). It was deproteinized immediately, and the protein-free clear supernatant evaporated to dryness *in vacuo* at a temperature not exceeding 55° C. The residue was taken up with about 10 ml water, and the solution thus obtained used for determination of both amines and amino acids.

For the determination of amino acids, the solution was once more dried *in vacuo*, and the residue taken up with 3 ml 75% ethanol. Two-dimensional chromatograms of two samples were run, using 0.2 ml of the final solution, according to Mizell & Simpson (1961).

For the determination of the amines, the solution was extracted by the method of Yoshinaga, Itoh, Ishida, Sato & Wada (1961), slightly modified (Neumark, 1964).

After three extractions with ethyl acetate (50 ml each time), the combined material was evaporated to dryness *in vacuo*, the residue taken up with 1 ml absolute methanol, and the solution thus obtained used for chromatography. Descending chromatograms were run using two 0.4 ml samples; the solvent mixture used was composed of n-butanol : glacial acetic acid : water (12:3:5). The dried chromatograms were treated with a solution of ninhydrin (0.2 g/100 ml acetone) containing 10% (v/v) glacial acetic acid. The sheets were dried at 110° C for 4 min.

For the determination of peptides, the epididymal semen was deproteinized by saturation with ammonium sulphate. After removal of the precipitate, the supernatant was dialysed against constantly flowing distilled water for 36 hr. The non-dialysable material was evaporated *in vacuo*, and the residue taken up with 75% ethanol. The solution thus obtained served for chromatography. About 2 ml was applied to a paper sheet and chromatographed in one direction. The different peptides were spotted by developing small reference strips of the chromatogram. The corresponding areas in the main chromatogram were cut out and eluted with 75% ethanol. After evaporating the ethanol, the peptides were hydrolysed for 22 hr at 110° C *in vacuo* and in the hydrolysate

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the amino acids determined with an automatic Beckman Amino Analyser. Molecular weight estimation of one of the major peptides was carried out by gel-filtration with Sephadex G-75.

The following results were obtained:

(i) Amino acids. Seventeen ninhydrin-reactive spots were detected. Twelve of these were identified as histidine, cysteic acid, arginine, aspartic acid, glycine, serine, glutamic acid, tyrosine, methionine, and components of the leucine group.

TABLE 1
AMINO ACID COMPOSITION OF THREE PEPTIDES EXTRACTED
FROM RAM EPIDIDYMAL SEMEN

Amino acid	$\mu\text{moles}/0.2 \text{ ml fluid}$		
	Peptide I (R_F 0.21)	Peptide II (R_F 0.29)	Peptide III (R_F 0.34)
Lysine	0.0034	0.0124	0.0068
Histidine	trace	trace	0.0025
Arginine	—	—	trace
Cysteic acid	—	trace	trace
Aspartic acid	0.0055	0.0234	0.0088
Threonine	0.0032	0.0187	0.0046
Serine	0.0192	0.0260	0.0124
Glutamic acid	0.0075	0.0247	0.0096
Proline	trace	trace	trace
Glycine	0.0160	0.0448	0.0159
Alanine	0.0069	0.0332	0.0128
Half cystine	—	—	—
Valine	trace	0.0090	0.0033
Methionine	—	0.0492	trace
Isoleucine	0.0010	0.0090	0.0035
Leucine	0.0020	0.0161	0.0061
Tyrosine	trace	—	trace
Phenylalanine	trace	—	trace

(ii) Amines. Three amines were detected, the R_F values of the two different samples being 0.79 (0.82), 0.25 (0.25) and 0.12 (0.13). The compound with the highest R_F value seems to resemble ephedrine. The compound with the lowest R_F value could be an imidazole derivative; in its behaviour on chromatograms it resembled histamine and related compounds.

(iii) Peptides. Four to six peptides were detected by one-dimensional paper chromatography (solvent system—*butanol:acetic acid:water* (12:3:5)); the amino acid composition of the three major peptides is given in Table 1. When a two-dimensional chromatogram was run, using *methyl-ethylketone:butanol:water* (2:2:1) as the second system, two additional spots were detected. The molecular weight of one of the three major peptides mentioned above was estimated by gel filtration to be about 3000.

The presence of a large number of free amino acids in ram epididymal semen raises the question of their possible role as a source of energy for the epididymal spermatozoa; it is known that the ram epididymal semen contains only very little reducing sugar (White & Wales, 1961), but that according to

Mann (1964) "free amino acids represent an extracellular oxidizable substitute for the aerobic metabolism of spermatozoa". The amines may perhaps be formed by decarboxylation of amino acids. Some peptides are known to be of physiological importance (Fruton & Simmonds, 1960), and it is not unlikely, therefore, that those present in epididymal semen may also be biologically active.

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