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Amino Acid Composition of Semen and the Secretions of the Male Reproductive Tract

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Abstract

The free amino acid content of rete testis fluid, epididymal fluid, vesicular fluid and ejaculated seminal plasma was studied in the ram, bull and rat, and a comparison made with the blood plasma of the same species. The amino acid content of human seminal plasma and the various fractions of the boar ejaculate was also determined.

Glutamic acid was found to be the predominant amino acid in the secretions of the reproductive tract of the ram and bull. It is present in high concentrations in the rete testis fluid of both species, and is further concentrated along the tract. In the rat glycine and alanine are the predominant amino acids in the rete testis fluid, while glutamic acid is the principal amino acid in epididymal fluid, as in the other species studied. Glycine is also the principal amino acid in human seminal plasma. The concentration of amino acids is greatest in the sperm-rich fraction of the boar ejaculate.

The breakdown of protein to amino acid was studied in ram and bull semen. This is at least partly due to enzymically induced changes.

Introduction

Although there are several reports on the free amino acid composition of ram and bull semen and reproductive tract fluids (Gassner and Hopwood 1952; Bhargava *et al.* 1959; Hopwood and Gassner 1962; Setchell *et al.* 1967), no direct comparison has been made between the amino acid content of the following fluids: rete testis fluid, epididymal plasma, vesicular fluid, seminal plasma and blood plasma. There are also little data available on the amino acid composition of the reproductive tract fluids of the rat or human. No published information for the boar had apparently appeared when these experiments were being undertaken; however, subsequently a paper by Johnson *et al.* (1972) came to the attention of the authors. The present study was carried out in an attempt to remedy these deficiencies.

After ejaculation there is a rapid decrease in the concentration of non-dialysable protein nitrogen and a simultaneous increase in non-protein nitrogen and free amino acids in human semen (Jacobsson 1950; Lundquist 1950; Mann 1964). In the present study the breakdown of protein to amino acids was studied in ram and bull semen.

Materials and Methods

Testicular fluid was collected from conscious rams and bulls by cannulation of the vasa efferentia using the techniques described by Voglmayr *et al.* (1966, 1969). Rete testis fluid was collected from a catheter placed in the rete testis of anaesthetized rats 12 h after ligating the vasa efferentia (Tuck *et al.* 1970). Semen was obtained from the bull by an artificial vagina and from the ram by an artificial vagina or electroejaculation (Blackshaw 1954). Boar semen was obtained in fractions using a dummy sow; human semen was collected from normal subjects and deproteinized within a few minutes.

Seminal vesicle and epididymal fluids were collected from the reproductive tracts of rams and bulls immediately after slaughter. Epididymal plasma was separated from the spermatozoa by centrifugation. Blood was collected from the jugular vein of rams and bulls and from the heart of anaesthetized rats; where possible blood was collected from the same animals that were sampled for reproductive fluids. All fluids were brought to 4°C immediately on collection and deproteinized with equal volumes of acetone.

Initially, one- and two-dimensional paper chromatography (Whatman No. 1 paper) was used to identify the amino acids present. One-dimensional papers were run in butanol–glacial acetic acid–water (12 : 3 : 5 v/v). Two-dimensional papers were run in butanol–acetic acid–water and then run at right angles in phenol–water–ammonia (160 : 40 : 1). The phenol and water solvent was kept as a stock solution and the ammonia added as required.

High-voltage electrophoresis (Shandon, model L24) was subsequently used to separate the amino acids. Samples were layered on to the origin lines on Whatman No. 1 chromatography paper using a graduated 10-μl Hamilton syringe; 10–100 μl were spotted, the samples being applied in 10-μl portions which were dried between successive applications. A formic acid–glacial acetic acid–water buffer (formic acid, 78 ml; glacial acetic acid, 148 ml; water up to 2·5 litres), pH 1·9, was used to separate lysine, arginine, glycine, alanine and serine (6 kV for 30 min). A pyridine–acetic acid–water buffer (pyridine, 25 ml; glacial acetic acid, 10 ml; water up to 2·5 litres), pH 5·3 (6 kV for 20 min), or a potassium hydrogen phthalate buffer (5·10 g potassium hydrogen phthalate and 0·868 g sodium hydroxide made up to 1 litre with distilled water), pH 5·9 (5 kV for 40 min), were used to separate glutamic and aspartic acids.

The amino acid spots on the electrophoretograms and chromatograms were developed by dipping the paper in 0·2% ninhydrin in acetone, with pyridine added as preservative. The papers were heated at 60°C for 22 min and the spots fixed using a saturated $Cu(NO_3)_2$ solution in acetone [1 ml $Cu(NO_3)_2$, 100 ml acetone, concentrated HNO_3 added dropwise till solution clear]. The amino acid spots on the electrophoretograms were quantitated by eluting the colour from each into 3 ml of 70% methanol. The samples were read at 510 nm in a Hitachi Perkin–Elmer spectrophotometer. This technique is accurate for amino acid concentrations between 0·025 and 0·100 mM; the standards and whenever possible the samples were spotted within this range. Standards used were 'Standard Solutions for Chromatography Set No. 1' (Shandon, London).

Accuracy is adversely affected when background colour is high, and care was taken to keep it as low as possible. There are significant differences between optical densities obtained from different sheets of paper and background for each paper was subtracted from each sample; it was kept to a minimum by developing all sheets from one sample on the same day and by maintaining the conditions of staining and colour development as constant as possible. As a further precaution, standard solutions were included on every electrophoretogram and the amino acid concentrations of unknown solutions were determined by reference to the standard on that particular sheet. The validity of this method for separation and quantitation of amino acids has been checked by Young *et al.* (1964). The results obtained by high-voltage electrophoresis have also been verified using an amino acid autoanalyser.

When six different fluids from the reproductive tract of rams and bulls were analysed by electrophoresis and the autoanalyser (Division of Food Research, CSIRO, Ryde, N.S.W.) the mean values (mM) were respectively: glycine 1·36, 1·39; alanine 0·98, 1·26; lysine 0·48, 0·54; arginine 0·37, 0·26; aspartic acid 0·74, 0·92; serine 1·73, 2·55; glutamic acid 6·10, 8·94.

The breakdown of protein to amino acids was studied in ram and bull semen and seminal plasma, incubated at 37°C. Aliquots were taken at zero time and after 3 h except for the time-course study of protein breakdown in seminal plasma, when samples were taken at zero time, 45 min, 1½ h and 3 h. Aliquots taken before and after incubation were deproteinized with six volumes of 95% ethanol and the supernatant was spun off, evaporated to dryness and reconstituted in distilled water. One millilitre of each sample was analysed in duplicate for total amino nitrogen by the ninhydrin colorimetric technique described by Rosen (1957). The standards were 0·1, 0·2, 0·3 and 0·4 μM glutamic acid (Univar) and both samples and standards were read against a reagent blank at 570 nm in a spectrophotometer.

The data in Table 2 have been subjected to analysis of variance and the difference between the mean of the control and treated samples was assessed by *t*-tests using the degrees of freedom associated with the interaction mean square from the analysis of variance.

Results

Amino Acid Values

The concentration of free amino acids in fluids from the reproductive tract of the ram, bull and rat and in boar and human seminal plasma is given in Table 1. The data presented here are for the seven amino acids which could be accurately separated and quantitated by high-voltage electrophoresis.

1. Ram. In ram rete testis fluid, glutamic acid and glycine were the predominant amino acids, with smaller quantities of serine, alanine, aspartic acid, arginine and lysine; some proline was also found with the amino acid autoanalyser. The concentration of almost all the amino acids was greater in fluid collected from the cauda epididymis and, with the exception of the dicarboxylic acids, still higher in the vesicular fluid. The amino acid concentrations in blood plasma were lower than in any of the fluids of the reproductive tract. Although the mean value for glutamic acid in blood plasma is comparatively high, the values fluctuate even within individual rams. A comparison of seminal plasma collected by electroejaculation and by an artificial vagina showed that the concentrations of alanine and lysine were similar, while there was less glycine, aspartic acid, glutamic acid and serine in plasma collected by the artificial vagina, but more arginine.

2. Bull. The concentration of all amino acids except alanine was considerably greater in epididymal plasma than in rete testis fluid. The glutamic acid content of epididymal plasma was particularly high and, as with the ram, it was the predominant amino acid in bull vesicular fluid and seminal plasma. Bull blood plasma also had a lower free amino acid content than the fluids of the male reproductive tract that were examined.

3. Rat. In contrast to the ram and bull, rete testis fluid collected from the rat had a low concentration of glutamic acid, and glycine was the predominant amino acid. However, the concentration of glutamic acid markedly increased in the epididymis and, as with the ram and bull, it was the principal amino acid in this fluid. The free amino acid content of rat blood plasma was lower than that of rete testis fluid; however, in epididymal fluid the concentrations were similar except for glutamic acid, which was considerably increased. No free amino acids could be detected in rat seminal vesicle fluid using the techniques of this study.

4. Human. Glycine was the principal amino acid in human seminal plasma; there was also a high concentration of glutamic acid and, in contrast to other species studied, a large amount of arginine.

5. Boar. The content of amino acids in boar seminal plasma increased as the sperm-rich fraction was ejaculated and this had the higher total concentration of amino acids, glutamic acid predominating. However, the amino acid content of the fraction collected between the pre-sperm fluid and sperm-rich plasma was low.

Incubation of Semen

When whole ram or bull semen was incubated for 3 h at 37°C the concentration of total amino nitrogen increased significantly from 12·7 to 19·4 mM in the ram ($t = 3{\cdot}07$, $P < 0{\cdot}05$, $n = 5$) and from 11·31 to 14·98 mM in the bull ($t = 3{\cdot}90$, $P < 0{\cdot}05$, $n = 5$).

Table 1. Concentrations of amino acids in fluids of the reproductive tract and blood plasma

Values are mean concentrations of amino acid (mM) ± S.E. Numbers of animals are given in parentheses

Species	Fluid	Alanine	Glycine	Arginine	Lysine	Glutamic acid	Aspartic acid	Serine
Ram	Rete testis fluid (4)	0·33±0·04	1·87±0·18	0·14±0·04	0·08±0·02	2·03±0·50	0·33±0·07	0·36±0·09
	Epididymal plasma (10)	0·84±0·13	1·36±0·19	0·55±0·14	0·55±0·15	12·21±0·93	0·89±0·13	1·66±0·27
	Seminal vesicle fluid (4)	1·93±0·36	3·89±0·67	1·11±0·13	0·98±0·17	2·27±0·30	0·41±0·10	4·06±0·84
	Seminal plasma (E.J.)[A] (4)	1·18±0·20	2·28±0·15	0·78±0·15	1·06±0·24	8·26±3·36	2·52±0·73	4·78±0·50
	Seminal plasma (A.V.)[A] (3)	2·35±0·77	1·45±0·25	1·17±0·06	1·02±0·02	4·53±0·28	1·30±0·54	2·78±0·28
	Blood plasma (4)	0·17±0·04	0·41±0·09	0·12±0·06	0·10±0·03	0·42±0·08	0·10±0·04	—[B]
Bull	Rete testis fluid (4)	1·04±0·16	1·09±0·16	0·33±0·13	0·16±0·04	2·19±0·24	0·43±0·12	0·73±0·10
	Epididymal plasma (8)	0·98±0·08	1·73±0·18	0·47±0·08	0·38±0·06	11·89±0·75	1·68±0·11	3·59±0·35
	Seminal vesicle fluid (5)	10·60±0·11	0·87±0·12	0·44±0·04	0·24±0·04	1·11±0·14	0·29±0·07	1·03±0·14
	Seminal plasma (6)	0·98±0·04	0·88±0·11	0·53±0·06	0·65±0·11	8·04±1·27	0·93±0·13	1·82±0·15
	Blood plasma (6)	0·21±0·02	0·25±0·06	0·12±0·02	0·05±0·02	0·16±0·03	0·10±0·01	—[B]
Rat	Rete testis fluid (8)	0·90±0·07	1·87±0·08	0·33±0·10	0·63±0·06	0·08±0·02	0·17±0·07	0·78±0·06
	Epididymal plasma (4)	0·22±0·07	0·48±0·08	0·07±0·04	0·05±0·03	0·76±0·07	0·25±0·12	0·64±0·09
	Blood plasma (9)	0·29±0·02	0·24±0·03	0·09±0·02	0·15±0·02	0·17±0·02	0·17±0·03	—[B]
Human	Seminal plasma (4)	2·25±0·26	7·41±1·91	5·15±0·56	1·68±0·78	6·49±0·46	2·24±0·32	4·07±0·58
Boar[C]	A. Pre-sperm fluid (3)	0·08±0·06	0·15±0·10	0·02±0·02	0·02±0·03	0·43±0·40	0·13±0·08	0·24±0·20
	B. Sperm-rich plasma (3)	0·23±0·06	0·53±0·22	0·08±0·02	0·06±0·02	5·90±1·77	0·49±0·14	0·47±0·13
	C. Plasma of B → D fraction (2)	0·51	0·67	0·05	0·02	0·76	0·31	0·41
	D. Post-sperm plasma (3)	0·20	0·21	0·02	0·02	0·45	0·27	0·65

[A] E.J. = electroejaculation; A.V. = artificial vagina.

[B] Serine could not be satisfactorily separated from valine in blood plasma.

[C] Samples collected from the boar were: A, plasma prior to sperm-rich fraction; B, plasma from sperm-rich fraction; C, plasma obtained between sperm-rich and post-sperm fractions; D, plasma from post-sperm fraction.

Over this incubation period the increase in total amino nitrogen in seminal plasma was not as great as that observed in the whole semen from which the plasma was prepared. Experiments using the seminal plasma showed that the rate of amino nitrogen formation decreased after the first 45 min, particularly in the case of the ram (Fig. 1). The addition of antibiotics (1 mg each of crystalline penicillin G and strepto-

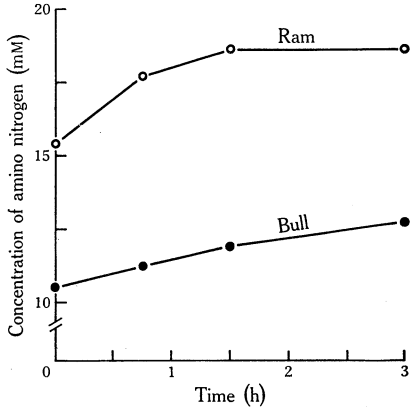

Fig. 1. Amino nitrogen production in seminal plasma over a 3-h incubation period at 37°C.

mycin sulphate) to the seminal plasma (1·0 ml) appeared to decrease the amount of amino nitrogen produced in 3 h, although this difference was not statistically significant, and when seminal plasma that had been heated for 5 min at 100°C was incubated there was little or no increase in amino nitrogen. This was more evident in the bull than in the ram, where the control values were variable (Table 2). It may be noted that the initial amino nitrogen content was greater in the pre-heated sample.

Table 2. Amounts of amino nitrogen produced during incubation of seminal plasma

Values shown represent changes in amino nitrogen concentration (mM) during incubation for 3 h of control seminal plasma, seminal plasma that had been heated to 100°C for 5 min and seminal plasma to which antibiotics (1 mg each of penicillin G and streptomycin sulphate) had been added. Values in parentheses are initial concentrations of amino nitrogen ± S.E.M. Mean values are given for five replicates

Species	Control plasma	Heated plasma	Plasma + antibiotics
Bull	2·22 (10·50±2·62)	−0·29* (12·37±2·47)	1·54 (10·30±2·36)
Ram	3·19 (15·36±5·09)	1·65 (20·58±4·76)	1·90 (14·88±3·25)

* Significantly less than control ($t = 4{\cdot}25$, $P < 0{\cdot}01$).

Discussion

The results reported here agree fairly closely with previous studies of testicular fluid. Thus Setchell *et al.* (1969) quoted a value of 1·93 mM for glutamic acid in the ram determined by enzyme methods, while a value of 1·13 mM was reported by Sexton *et al.* (1971) for the bull determined with a Beckman model 120C amino acid autoanalyser.

Although rat rete testis fluid was collected after ligation of the vasa efferentia for 12 h, Tuck *et al.* (1970) have shown that this has remarkably little effect on testicular vascular function, as measured by capillary blood flow and permeability, or on tubular function as measured by rubidium uptake. Also there was no change in the electrolyte composition of rete testis fluid after release of the ligature. One may infer therefore that there would be little change in amino acid synthesis as a result of ligation.

The amino acids in rete testis fluid are apparently synthesized from blood glucose in the seminiferous tubules, since after infusion of [U-^{14}C]glucose radioactivity can be detected in the amino acids of the rete testis fluid of the ram, and particularly in glutamic acid (Setchell *et al.* 1967). There also appears to be an inverse relationship between the glucose and the glutamic acid concentration in rete testis fluid and a direct correlation with the number of spermatozoa present.

The testicular origin of at least some of the amino acids in bull seminal plasma has been shown earlier (Gassner and Hopwood 1952; Hopwood and Gassner 1962) and, despite the contribution of the seminal vesicles, the concentration of most amino acids in the seminal plasma is not altered significantly by removal of these glands (Hess *et al.* 1960). However, in the boar the seminal vesicles appear to contribute most of the glycine to the seminal plasma (Johnson *et al.* 1972).

The concentration of all amino acids except glycine was greater in ram and bull epididymal fluid than in rete testis fluid due presumably to water reabsorption in the caput epididymis. If the increase in amino acid concentration was due merely to reabsorption of fluid in the epididymis, the ratio of the concentration of each amino acid in rete testis fluid : epididymal plasma would remain the same. This is clearly not the case and the ratio is highest for glycine and lowest for glutamic acid; it appears therefore that certain amino acids, particularly glycine, are selectively reabsorbed or metabolized within the epididymis or both. The evidence suggests that glycine is reabsorbed in the caput epididymis along with water, sodium and chloride ions (Scott *et al.* 1963; Wales *et al.* 1966). Perhaps the mechanisms for reabsorption of amino acids in the epididymis are similar to those operating in the renal tubule (Young and Freedman 1971). One system transports the dibasic acids (lysine, arginine, ornithine, and possibly histidine and cystine—although a separate pathway has been postulated for cystine). A second mechanism transports the acidic amino acids, glutamic and aspartic acid, and a third the neutral amino acids except glycine, which is transported by a fourth mechanism.

There is a high concentration of aspartic acid in ram seminal plasma which cannot be accounted for by the secretions of the seminal vesicles. It may originate in the prostate, since a high concentration of free amino acids has been reported in the prostatic secretion of the dog, rabbit and rat (Barron and Huggins 1946; Awapara 1952) which Mann (1964) suggests is the outcome of the combined action of proteolytic and transaminating enzymes in the glandular tissue. Aspartate aminotransferase has been found in ram seminal plasma. It occurs to some extent in epididymal fluid (Alumot and Schindler 1965; Murdoch and White 1968) and its activity may also be partly responsible for the high concentration of aspartic acid in ram seminal plasma.

The results reported here suggest that the increase in amino nitrogen that occurs on incubation of semen after ejaculation is at least partly due to enzymically induced changes. There are clearly species differences in the rate of breakdown of seminal protein to amino acids. Thus the concentration of free amino acids in the human

ejaculate increases rapidly up to about 200 mg amino nitrogen/100 ml on collection, due to enzymes present in the prostatic secretion (Lundquist 1953) but protein breakdown in ram and bull semen is much slower.

The significance of the high amino acid concentration in the fluids of the male reproductive tract is difficult to assess at present. It has been suggested that ejaculated bull spermatozoa are able to incorporate amino acids into acrosomal protein (Bhargava *et al.* 1959) but subsequent investigations suggest that this was due to contaminating bacteria (Setchell *et al.* 1967). Recently, testicular and ejaculated ram spermatozoa have been shown to oxidize both [U-^{14}C]glutamic acid and leucine to a limited degree but neither substrate stimulated respiration above endogenous levels (Setchell *et al.* 1967). Amino acids may be necessary for nucleic acid synthesis within the seminiferous tubule, as a number of amino acids are involved in the synthesis of purine and pyrimidine bases. For example, glycine has been shown to play a central role in pyrimidine synthesis, and glutamic and aspartic acids are purine precursors (Buchanan 1960; Crosbie 1960).

There is also some evidence that amino acids in seminal plasma may assist in sperm survival, since excessive dilution of semen, which had a deleterious effect on spermatozoa, can allegedly be partly counteracted by including amino acids in the diluting medium (see Mann 1964). Amino acids may act by virtue of their ability to bind certain heavy metals which are present in the usual salt solution diluents in trace amounts, and which are toxic to the spermatozoa.

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