PRELIMINARY NOTES

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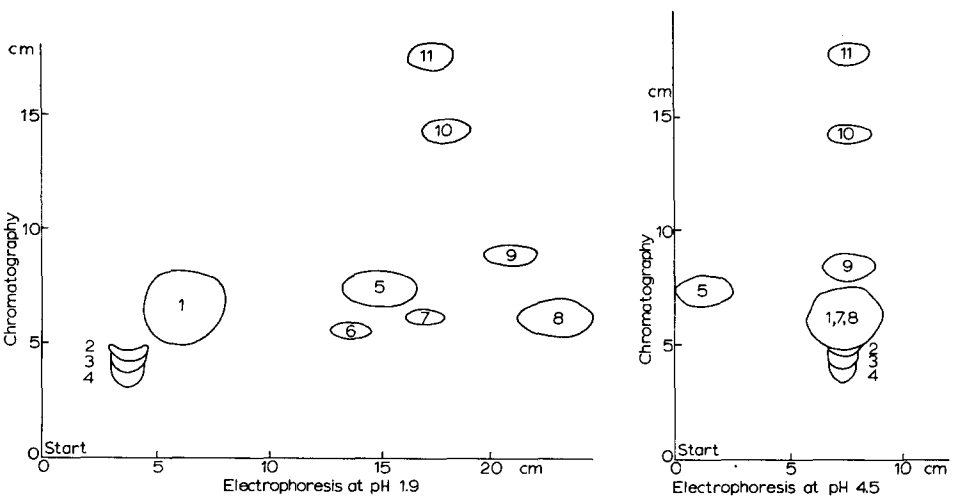
The occurrence of hypotaurine and other sulfur-containing amino acids in seminal plasma and spermatozoa of boar, bull and dog

In connection with an examination of a possible relationship between the composition of semen and fertility, an investigation was carried out into the amino acid composition of boar seminal plasma. The free amino acid composition of bull and boar semen has been given by KUBICEK, LINDNER AND SANTAVY[1] and MANN[2]. An adequate quantity of boar semen (usually 5 ml) was deproteinized with trichloroacetic acid and, after centrifuging, the liquid was passed through a column of Dowex 50-W in the H^+ form. The amino acids were eluted with 2 N or 6 N ammonia and, after evaporation of the ammonia, the amino acids were examined by electrophoresis at pH 4.5 and at pH 1.9 followed by chromatography in the solvent system butanol–acetic acid–water (12:3:5, by vol.) as described earlier[3,4]. The amino acids were detected with ninhydrin; the patterns are presented in Figs. 1 and 2.



Figs. 1 and 2. Electrophoresis at pH 4.5 and at pH 1.9 followed by chromatography in the mixture butanol–acetic acid–water (12:3:5, by vol.) of boar seminal plasma after freeze-drying. 1, hypotaurine; 2 and 3, sulfur-containing amino acids, possibly thiotaurine and *S*-sulfocysteamine, respectively; 4, taurine; 5, glutamic acid; 6, asparagine; 7, serine; 8, glycine; 9, alanine; 10, valine; 11, leucine.

Leucine, phenylalanine, valine, alanine, glycine, serine and glutamic acid are present in readily detectable amounts; basic amino acids were seldom present and, if so, only in very small quantities. However, the most striking phenomenon is a very large spot of an amino acid, which, after electrophoresis at pH 4.5, appears in

the neutral fraction approximately in the same position as glycine, but, after electrophoresis at pH 1.9, far to the left of glycine. It follows that this amino acid must contain, besides the amino group, an acidic group stronger than the carboxyl group, *e.g.* a sulfonic acid group. Taurine was therefore subjected to both electrophoresis and chromatography. After electrophoresis at pH 4.5 it appeared in the neutral group and on electrophoresis at pH 1.9 it moved far to the left, even somewhat further than the unknown amino acid (the position is indicated in Fig. 2).

Small amounts of the amino acid were prepared by preparative electrophoresis[4]. Microreactions according to FEIGL[5] revealed the presence of a sulfinic acid or a sulfonic acid group.

Italian investigators[6] have reported the chromatographic behavior of eight sulfur-containing amino acids, all related to cysteine. From its position in the neutral group after electrophoresis (pH 4.5) it would appear that the unknown amino acid cannot be cysteic acid, cysteine sulfinic acid, alanine thiosulfonic acid or *S*-sulfocysteine, because all these compounds contain two acidic groups and, after electrophoresis at pH 4.5, they should appear to the left of glutamic acid. Taurine is also excluded, as already mentioned; the three remaining amino acids are hypotaurine, thiotaurine and *S*-sulfocysteamine.

The presence of hypotaurine has been demonstrated by the following tests: (a) Spraying with $KMnO_4$–H_2SO_4 resulted in an immediate discoloration. (b) Elution from a column Dowex 50-W in the Na^+ form was not possible with the formate buffer composed of 950 ml of 1 N HCOOH and 50 ml of 1 N NaOH, but succeeded with a more alkaline formate buffer composed of 750 ml of 1 N HCOOH and 250 ml of 1 N NaOH. This is in accordance with the observations of MOSTI, DE MARIA AND DE MARCO[7]. (c) Oxidation with H_2O_2 or with Norit resulted in the formation of taurine. From all these experiments it may be concluded that the unknown amino acid must be hypotaurine.

In order to be sure that hypotaurine is not formed during the experimental procedure, a quantity of seminal plasma was freeze-dried and the alcoholic extract of the residue was immediately submitted to electrophoresis at pH 4.5 followed by chromatography. The normally occurring amino acids were present and not only did a large spot of hypotaurine appear but in addition three spots close to and below hypotaurine (the spots 2, 3 and 4 in Fig. 1). Compound 2 gave a blue color with ninhydrin, Compound 3 an orange-brown color and Compound 4 a purple color. All these compounds moved to the left on electrophoresis at pH 1.9, equally as far as taurine. None reacted with the $KMnO_4$–H_2SO_4 solution. Sulfur could be shown to be present in all these compounds. They may possibly be thiotaurine, *S*-sulfocysteamine and taurine, but until now the quantities which could be prepared have been too small to be entirely sure of this. These points are being further examined.

The question arises whether hypotaurine is present only in the seminal plasma or also in the spermatozoa. In order to gain further information on this point, the ejaculate was collected in three fractions. In one experiment the first fraction was about 20 ml and did not contain any appreciable quantity of living spermatozoa; amino acids appeared to be absent. The second fraction was 65 ml and the concentration of the spermatozoa was 1068×10^6 per ml; it contained a considerable quantity of amino acids with hypotaurine as the main component. The third fraction was 250 ml and contained 40×10^6 spermatozoa per ml and only a small quantity of

amino acids. This shows that the hypotaurine of seminal plasma is associated mainly with the sperm-rich fraction of the ejaculate.

In order to investigate the possibility that the spermatozoa themselves contain hypotaurine, 80 ml of the second fraction of an ejaculate were centrifuged and the spermatozoa were washed with the normal diluent for semen. (1 l aqueous diluent contained 20.0 g sodium citrate, 2.1 g $NaHCO_3$, 0.4 g KCl, 3.0 g sulfanilamide, 3.0 g glucose, 1.0 g dihydrostreptomycin and 10^6 I.U. penicillin G, sodium salt.) To a quarter of the washed spermatozoa 20 ml of this diluent was added and 5 ml of the mixture was examined as described above; it appeared that practically no amino acids were present. The remaining spermatozoa were treated in an Elvehjem–Potter homogenizer; one part with the diluent, the second part with water and the third part with 50 % alcohol. Immediately similar amounts of the mixtures were examined. Relatively large amounts of hypotaurine and much smaller quantities of other amino acids were observed, especially on treatment with water and 50 % alcohol. From these experiments it can be concluded that spermatozoa contain hypotaurine and that this is liberated quickly on treatment with water and slowly on treatment with the diluent. As hypotaurine is always present in boar seminal plasma the question remains when and where it is liberated *in vivo*. Further investigations are in progress in order to investigate the metabolism of hypotaurine, in which part of the spermatozoa it occurs, and the role it plays in the sperm. Similar experiments have been carried out with seven boars and the results were fairly similar.

Finally, similar chromatograms have been made of the seminal plasma of the bull and dog. Hypotaurine could always be demonstrated, but it was not the main amino acid. The three other sulfur-containing amino acids were often present in relatively greater amounts. Other differences were also apparent, *e.g.* in bull seminal plasma alanine and glutamic acid predominated and basic amino acids were always present, whilst in boar seminal plasma basic amino acids could seldom be seen. As to the data cited by MANN, leucine could often very clearly be shown in bull seminal plasma, but the concentration varied considerably. It is possible, therefore, that it could not be detected by BHARGAVA *et al.* (cited by MANN). In dog seminal plasma the concentrations of all the amino acids were very low.

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1 R. KUBICEK, E. LINDNER AND F. SANTAVY, *Bull. Soc. Chim. Biol.*, 41 (1959) 1345.
2 T. MANN, *Biochemistry of Semen and of the Male Reproductive Tract*, Methuen, London, 2, 1964, p. 97.
3 C. J. G. VAN DER HORST, *Nature*, 191 (1961) 73.
4 C. J. G. VAN DER HORST, *Nature*, 196 (1962) 147.
5 F. FEIGL, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 6, 1960, p. 263.
6 C. DE MARCO, R. MOSTI AND D. CAVALLINI, *J. Chromatog.*, 18 (1965) 492.
7 R. MOSTI, V. DE MARIA AND C. DE MARCO, *Ital. J. Biochem.*, 14 (1965) 107.

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