The Occurrence of Phenylalanine- and α-Aminocaprylic Acid-α-Ketoglutarate Transaminase in Boar Spermatozoa

Earlier[1,2] it has been shown that phenylpyruvic- and *p*-hydroxyphenylpyruvic acid are sometimes present in boar semen with a low fertilizing ability. These acids are formed by transamination of phenylalanine and tyrosine[2]. In this communication more data about these transaminases will be reported.

Material and method. In all experiments spermatozoa have been obtained by centrifuging the ejaculate at 3500 *g* for 10 min. The spermatozoa are washed twice with 5% sucrose solution and centrifuged again. The precipitate is treated with phosphate buffer (pH 7.6) in a sonifier and the solution obtained after centrifugation is tested for transaminase activity or is used for preparation of acetone powder.

The transaminase activity is examined by incubation of the phosphate containing solutions with α-ketoglutaric acid and phenylalanine at 38 °C for $1^1/_2$ h. The keto acids are investigated by thin-layer chromatography, after which they are converted in amino acids and identified as such[3].

The acetone powder is obtained by addition of acetone to the above-mentioned phosphate containing solutions. The precipitate is washed with acetone and dried in a desiccator.

Results and discussion. (A) Incubation experiments of phenylalanine and the phosphate containing solutions with 3 different keto acids, viz. pyruvic acid, α-ketoglutaric acid and oxalo acetic acid, revealed that α-ketoglutaric acid was most effective in formation of phenylpyruvic acid. Pyruvic acid was quite ineffective, but incubation with oxalo acetic acid resulted in formation of small amounts of phenylpyruvic acid. Most probably this is due to the presence of small amounts of α-ketoglutaric acid, which are formed after addition of oxalo acetic acid. After incubation with α-ketoglutaric acid rather large amounts of glutamic acid are formed. Phenylalanine + α-ketoglutaric acid $\rightleftarrows$ phenylpyruvic acid + glutamic acid.

Surprisingly, in most cases a second keto acid is formed in addition to phenylpyruvic acid. After reduction with hydrogen it is converted into an amino acid with a Rf value higher than that of leucine. (The unknown amino acid will be indicated further as B). B is lying on the chromatograms on the produced part of the line leu, val, ala and gly. This suggests that B may be an homolog of leucine, for instance α-aminocaprylic acid.

(B) Incubation experiments of the phosphate containing solutions, to which now α-ketoglutaric acid has been added, with different amino acids, viz. leucine, valine, glycine, alanine, tyrosine, α-aminobutyric acid, β-alanine and α-aminocaprylic acid, showed that tyrosine and α-aminocaprylic acid and to a less degree leucine are transaminated. The other amino acids do not react. In all cases the formation of the corresponding keto acids could be demonstrated and the keto acid formed after incubation with α-aminocaprylic acid was seemingly the same as the unknown α-keto-B. This points also to the probability that the amino acid B must be α-aminocaprylic acid. The amino acid B can be obtained by boiling the spermatozoa with hydrochloric acid.

(C) Incubation experiments showed that the acetone powder has the same properties as the spermatozoa in all respects, even for the formation of α-keto-B. This would imply that the amino acid B must occur in the acetone powder. After hydrolysis with 6 *N* HCl, the presence of B could be shown.

(D) Finally, the presence of phenylalanine-α-ketoglutarate transaminase is confirmed, using the assay of SCANDURRA and CANNELLA[4], in which they determined the phenylpyruvic acid. In alkaline media this compound absorbs strongly with a maximum at 318 nm. The absorbance is read against a zero time blank containing all reagents but with NaOH added before α-ketoglutaric acid in order to prevent transamination.

We investigated 1 ml seminal plasma, 1 ml semen and the spermatozoa obtained after centrifugation of 0.5 ml semen. The spermatozoa were treated in a sonifier with 1 ml of phosphate buffer. In the Table the results are reported from 3 ejaculates of 3 boars. From the fertility records of the A.I. station, boar No. 1 and No. 2 were of normal fertility. Boar No. 3 was classified as having very low fertility. It is evident that normal boar seminal plasma has but very slight transaminase activity. The spermatozoa, however, do show a considerable transaminase activity. (An extinction of 2.0 corresponds with a conversion of 0.5 μ*M* phenylalanine during incubation of 20 μ*M* phenylalanine in 30 min at 38 °C.) The seminal plasma of boar No. 3 shows a rather large transaminase activity.

With the aid of this method, it seems possible to obtain information about the quality of the semen within 2 h. Field experiments in cooperation with the department of artificial insemination are going on. The results and a detailed description of the experiments will be given soon[5].

Boar No.		Date 9.5.1969	19.5.1969	23.5.1969
1	Seminal plasma	0.08	0.10	0.09
	Semen	0.08	0.28	0.14
	Spermatozoa	1.98	1.98	1.95
2	Seminal plasma	0.09	0.02	0.04
	Semen	0.31	0.62	0.15
	Spermatozoa	1.85	1.98	1.78
3	Seminal plasma	0.36	0.19	0.21
	Semen	0.49	0.34	1.50
	Spermatozoa	1.80	1.82	2.50

Transaminase activity (expressed as the extinction at 318 nm) after incubation of 1 ml seminal plasma, 1 ml semen and of spermatozoa occurring in 0.5 ml semen with 20 μ*M* phenylalanine and 20 μ*M* α-ketoglutaric acid during 30 min at 38 °C. The spermatozoa are treated in a sonifier with 1 ml phosphate buffer.

Zusammenfassung. Phenylalanin- und α-Aminocaprylsäure-α-Ketoglutarsäure-Transaminase konnte in den Spermatozoen des Ebers nachgewiesen werden. Mittels Aktivitätsbestimmung nach SCANDURRA und CANELLA[4] kann bereits nach 2 Stunden eine Qualitätsaussage über das Sperma gemacht werden.

C. J. G. VAN DER HORST

Laboratory of Biochemistry of Reproduction, Clinic of Veterinary Obstetrics and Gynaecology, University Centre 'De Uithof', Utrecht (The Netherlands), 20 August 1969.

[1] H. J. G. GROOTEN, Thesis, University of Utrecht (1967).
[2] C. J. G. VAN DER HORST, Proc. VIe Congr. de réproduction et insémination artificielle *11*, 1251 (1968).
[3] C. J. G. VAN DER HORST, Tijdschr. Diergeneesk. *90*, 1305 (1965).
[4] R. SCANDURRA and C. CANNELLA, Analyt. Biochem. *27*, 253 (1969).
[5] The author is indebted to Mr. D. SCHAKELAAR for his valuable technical assistance.