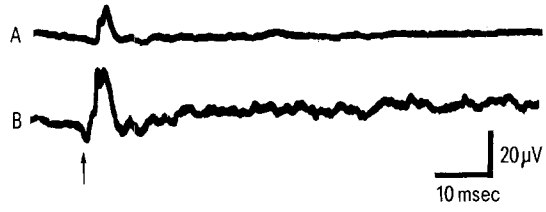

Fig. 2. Simultaneous recordings of the action potential on the nerve of the lateral canal ampullae (A) and of the evoked potential in the lateral ventral vestibular nucleus (B). The time interval which separates the 2 components of the potentials does not increase. The arrow indicates the beginning and the end of the injection.

The bimodal form of the potential poses a problem of interpretation that anatomico-functional correlations by histo-physiological methods will perhaps enable us to solve. Indeed, we know that the epithelium of the crista ampullaris contains 2 populations of sensory cells (HC I and HC II) afferented in a non-problematical way[3] by fibres of different calibers (1–12 μm)[16]. The 2 peaks of the action potential are therefore perhaps due to the mode of functioning of these cells.

1 Supported by the D.G.R.S.T. grant 77.7.0669 and I.N.S.E.R.M. (A.S.R. No. 6, grant 011).
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A method for the determination of glutamate oxaloacetate transaminase in sperm plasma from boars and bulls[1]

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Summary. A method is described for the determination of glutamate oxaloacetate transaminase (GOT) activity in sperm plasma from boars and bulls as a test for fertilizing ability.

GOT (E.C.2.6.1.1) activity in sperm plasma can be used as a criterion for semen quality and fertility[2–6]. Because existing colorimetric methods lack selectivity[7–9], and the coupled reaction assay for determination in blood serum (ultimately a measurement for NADH oxidation at 340 nm)[8,10] proved to be unsuitable for use in sperm plasma, we modified the Boehringer UV method[8].

Material and methods. 0.1 M Na/K phosphate buffer, pH 7.6; 0.58 M Tris, pH 10.85; trichloroacetic (TCA) solutions of 25- and 2.5% (w/v) in aqua dest.; solutions of 40 mM L-aspartate, 125 mM α-oxoglutarate and 2.4 mM NADH in phosphate buffer; malate dehydrogenase (MDH), diluted just before use to 250 μg/ml in phosphate buffer. All chemicals were of reagent grade. Absorbances were measured on a Shimadzu UV 200 spectrophotometer in 1-cm path length cells. Aqua dest. was used as a blank.

Sperm plasma was prepared by centrifugation of undiluted or with current buffers diluted[10] sperm for 15 min at 3500 × g. The supernatant was centrifuged once more and the resulting sperm-free supernatant diluted with phosphate buffer, 1:50 for bulls and 1:4 for boars, based on the original sperm volume. Then 1-ml samples of these dilutions were mixed with 2 ml aspartate and 0.2 ml oxoglutarate and incubated in stoppered polyethylene tubes for 30 min at 37 °C (L-aspartate + α-oxoglutarate $\overset{\text{GOT}}{\rightleftharpoons}$ L-glutamate + oxaloacetate). Next 0.5 ml 25% TCA was added to the incubation mixture under cooling at 0 °C. Centrifugation for 10 min at 8000 × g and, if necessary, filtration through 'Blaubandfilter no. 589[3] from Schleicher & Schüll' gave a clear and colourless solution for the subsequent MDH reaction (Oxaloacetate + NADH + $H^+ \overset{\text{MDH}}{\rightleftharpoons}$ L-malate + NAD^+).

For this purpose 2 ml of this solution was mixed with 0.8 ml Tris and 0.1 ml NADH (final pH 7.4–7.6). The absorbance at 340 nm and 25 °C was measured (A_1), 0.1 ml MDH was added and after 5 min the absorbance was measured again (A_2).

In case $A_2 < 0.15$ (due to a relatively high initial concentration of oxaloacetate) another 0.1-ml portion of NADH was added and after 5 min the new absorbance was read (A_2^1). In this case also a reference solution of 0.9 ml Tris, 2 ml 2.5% TCA and 0.1 ml NADH was measured (A_1^1). Absorbance values A_1 and A_2^1 were corrected to a reaction volume of 3 ml. Defining the GOT unit as the amount that catalyses the formation of 1 μmole of oxaloacetate per min at 37 °C under our conditions, it follows that GOT activity = $A \times d \times 185/\varepsilon$ units per ml of undiluted sperm plasma, in which $\Delta A = A_1 - A_2$ (or $A_1 + A_1^1 - A_2^1$), d = dilution factor of the original sperm plasma (50 or 4) and ε = molar absorption coefficient for NADH[7] at 340 nm = 6.22×10^3. If desired, the results may be related to the number of spermatozoa per ml of undiluted sperm, determined e.g. by Coulter Counter measurements.

Results and discussion. With diluted sperm plasma, linearity of the progression-with-time curve for the GOT reaction was established. Moreover, the relationship between ΔA and standard quantities of GOT and also between ΔA and oxaloacetic acid quantities, having been subjected to the whole and 2nd half of the reaction procedure respectively, proved to be linear. The reproducibility of measurements of 1 ejaculate is shown in the following table:

	n	ΔA (mean)	SD	Coefficient of variation
Sperm plasma bulls, 50× diluted	6	0.2488	±0.0038	1.5%
Sperm plasma boars, 4× diluted	6	0.2880	±0.0046	1.6%

The existing Boehringer coupled reaction assay[8] was split up into 2 separate reactions because of difficulties encountered in our initial experiments with sperm plasma. Some plasmas, especially when diluted with certain buffers, showed own absorption at 340 nm and/or developed turbidity when treated with Boehringer's reagent mixture. Besides, unwanted influence on ΔA determination may be caused by the presence of lactate dehydrogenase and glutamate dehydrogenase[10,11]. The described TCA treatment immediately stops the initial GOT reaction, yielding a deproteinized and clear solution. Under these conditions oxaloacetate proved not to be decomposed. After this, Tris was added to restore a favourable pH environment for the 2nd, MDH catalysed, reaction. Furthermore undiluted or slightly diluted sperm plasma appeared to be an adsorbent for oxaloacetate, thus partly inactivating this reaction component in the MDH reaction. This holds especially for bull sperm. Preliminary dilution of sperm plasma with phosphate buffer (50 times for bulls and 4 times for boars) eliminated this and other problems, connected with unwanted shifts of reaction equilibria when using undiluted or only slightly diluted plasma samples. Under these circumstances it proved to be desirable to raise the temperature and to lengthen the duration of the GOT reaction (respectively to 37 °C and 30 min) in order to produce enough oxaloacetate for a reliably sensitive measurement of the NADH decrease. For the ΔA measurements a reaction time of 5 min appeared to be sufficient for a complete conversion of oxaloacetate.

1 Publication A-336 of the Research Institute for Animal Husbandry Schoonoord.
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