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Note

Determination of free choline in human semen using an isotachophoretic analyser

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Free choline has been shown to be present in considerable amounts in the semen of animals. Various chemical tests have been used for the identification of seminal fluid and stains. Florence [1] developed a microscopic method by crystallizing seminal choline [2, 3], but these classical methods are no longer widely used. Some chromatographic tests for seminal choline have been reported more recently [4—6], as have an enzymatic method [7] and an enzymic fluorometric method [8] for the detection of free choline in semen.

This paper describes a new method for the measurement of free choline in semen by isotachophoretic analysis [9—13]. This method is compared with the enzymatic method based on the reaction of choline oxidase with choline, and the two sets of results agree well. The isotachophoretic method presented here has several advantages over previously described techniques in the medico-legal field.

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EXPERIMENTAL

Materials and methods

Normal human semen, saliva, vaginal fluid, blood, serum, urine and breast milk were collected from healthy individuals. Each sample was mixed with 3 volumes of 2% trichloroacetic acid and deproteinized by centrifuging at 1600 *g* for 10 min. The supernatant was applied to a column containing 10 ml of Diaion SK-1 (H^+-form of sulphonated cation exchanger, 100 mesh, Mitsubishi Kasei, Tokho, Japan), washed with deionized water, 40 ml of 0.2 *M* hydrochloric acid and 10 ml of 2 *M* hydrochloric acid, and then eluted with 20 ml of 2 *M* hydrochloric acid. The eluate was dried under reduced pressure and aliquots of the residue were analysed using an isotachophoretic analyser.

The enzymatic determination of free choline in samples was performed by using the method of Suzuki et al. [7].

Apparatus

The capillary apparatus was a Shimadzu IP-1B isotachophoretic analyser (Shimadzu, Kyoto, Japan). The determination of free choline in the semen was carried out in a capillary tube (20 cm × 0.5 mm I.D.) maintained at a constant temperature of 20°C. The detector cell was 0.05 × 0.5 mm I.D. The migration current was 100 μA. The leading electrolyte was 0.01 *M* potassium acetate, titrated with 17 *M* acetic acid to pH 4.0. The terminating electrolyte was 0.01 *M* carnitine chloride. The chemicals used were of analytical grade.

RESULTS AND DISCUSSION

The determination of free choline in semen has generally been performed by an enzymatic method using choline oxidase. We have reported in previous papers [11—13] that several compounds in biological samples which have an anion or cation group in the structure of the compound can be determined by isotachophoretic analysis. We have therefore tried to detect free choline in semen as a cation in the structure.

The leading electrolyte was 0.01 *M* potassium acetate titrated with acetic acid to pH 4.0 as described under *Materials and methods*. Carnitine chloride was used as the terminating electrolyte. Isotachophoretic runs of authentic choline (A), a semen sample (B), and a mixture of authentic choline and a semen sample (C) are shown in Fig. 1. Authentic choline is easily separated under the conditions used (Fig. 1A). The zone in the semen sample that has the same potential gradient as the zone of authentic choline was made to overlap by adding authentic choline to the semen sample, resulting in an elongation of the zone of choline in the semen sample (Fig. 1C). This fact indicates that the elongated zone is the zone of choline. The slope of the standard curve drawn by plotting the zone length against different concentrations of authentic choline was linear from 0 to 50 nmol.

It was possible to detect 1 nmol of choline in semen samples by using an isotachophoretic analyser under the analytical conditions described under *Materials and methods.*

In order to check the recovery of choline in the Diaion SK-1 concentration

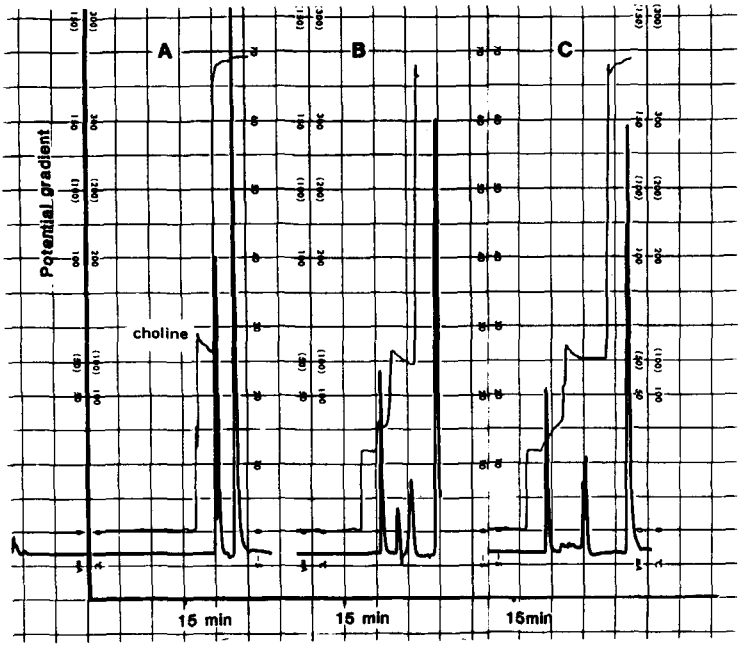

Fig. 1. Isotachophoresis of authentic choline (A), a semen sample (B), and a mixture of authentic choline and a semen sample (C). Leading electrolyte, 0.01 *M* potassium acetate titrated with acetate to pH 4.0; terminating electrolyte, 0.01 *M* carnitine chloride; migration current, 75 μA; chart speed, 10 mm/min.

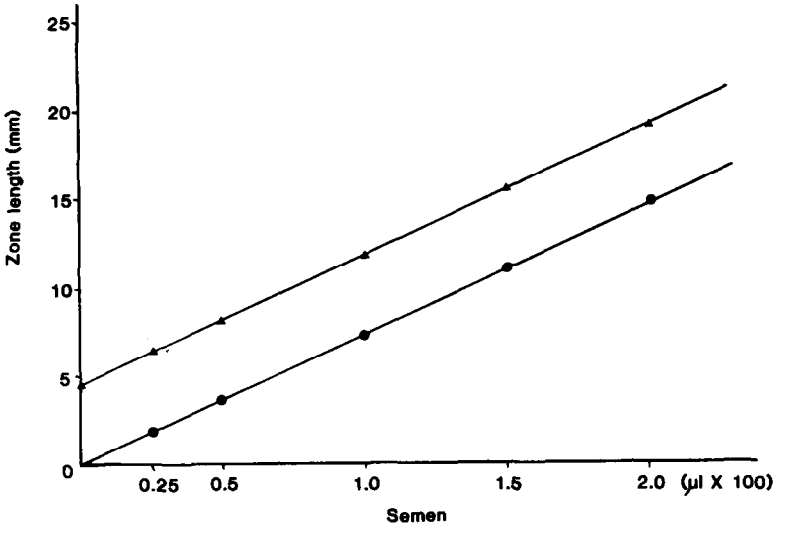

Fig. 2. Isotachophoretic detection of authentic choline in the presence of different amounts of semen: •—•, without choline addition; ▲—▲, with the addition of 10 nmol choline. Analytical conditions as in Fig. 1.

step, an internal standard of authentic choline (10 nmol) was added to the semen sample before chromatography. The same semen without any addition of choline was processed in parallel. The results indicated a recovery of the added choline ranging from 95 to 100% (Fig. 2).

TABLE I

COMPARISON OF CHOLINE CONTENTS IN HUMAN SEMEN DETERMINED BY ISOTACHOPHORESIS AND THE ENZYMATIC METHOD

Case No.	Choline content (mg/ml)	
	Isotachophoresis	Enzymatic method
1	1.688	1.626
2	1.668	1.618
3	1.807	1.746
4	1.668	1.631
5	1.668	1.618
6	1.807	1.750
Mean ± S.D.	1.721 ± 0.067	1.664 ± 0.064

A comparison of the determination of choline in semen samples by isotachophoretic analysis and by the enzymatic method is shown in Table 1. The two methods gave almost identical values for the choline contents of semen: isotachophoresis, 1.721 ± 0.067 mg/ml; enzymatic method, 1.664 ± 0.064 mg/ml. The determination of choline in various human body fluids (vaginal fluid, saliva, blood, serum, urine and breast milk) by isotachophoresis was also carried out, but it could not be detected in any other body fluids of human origin except semen.

Under the analytical conditions described here, γ-aminobutyric acid, polyamine in biological samples were also detected, as reported in a previous paper [14], but these compounds could be separated easily from choline. These results described above indicate that this method can be adequately used for the quantitative estimation of choline in semen. Isotachophoretic analysis is simpler than the enzymatic method reported in previous papers, and therefore should be very useful for the determination of choline in the medico-legal field.

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