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Note

Simple assay method using isotachophoresis for taurine in tissues of the rat

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Taurine is known to be present in almost all organs of animals, but the rôle played by this compound is not fully understood. The determination of this compound has been achieved by using a colorimetric method[1] and an amino acid analyzer after separating taurine by column[2], thin-layer[3] and high-voltage paper electrophoresis[4]. Recently, fluorometric[5,6], enzymatic[7] and radiometric[8] methods have been reported. However, these methods require time-consuming pretreatment of the sample.

We have devised a new simple and rapid method for detecting taurine in various organs of the rat. This isotachophoretic method has several advantages over previously described techniques.

EXPERIMENTAL

Male rats weighing 120 g were killed by decapitation. The serum and several tissues were kept frozen at −20°C if not analyzed immediately. Each tissue was homogenized in three volumes of water, an equal volume of 2% sulphosalicylic acid was added and the mixture was centrifuged at 1400 *g*.

The supernatant was applied on a column containing 10 ml of Diaion SK-1 and washed with deionized water. The effluent and washing were combined, transferred to a column containing 10 ml of Diaion SA-100, washed with deionized water and eluted with 2 *M* acetic acid. The eluate was dried under reduced pressure. The residue was subjected to high voltage paper electrophoresis[9] for detection of taurine and analyzed on an isotachophoretic and amino acid analyzers (Hitachi Model 835 liquid chromatograph).

Apparatus

The capillary apparatus was a Shimadzu IP-1B isotachophoretic analyzer[10,11] (Shimadzu, Kyoto, Japan). The determination of taurine 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 had an I.D. of 0.5 mm and length 0.05 mm. The migration current was 75 μA. The leading electrolyte consisted of 0.01 *M* HCl and amediol, pH 6.50. The terminating electrolyte was 0.01 *M* γ-aminobutyric acid and $Ba(OH)_2$, pH 10.90. The chemicals used were of analytical grade.

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RESULTS AND DISCUSSION

It was possible to detect taurine in several tissues and serum of the rat after partial separation. A standard curve drawn by plotting the zone length against different concentrations of the standard taurine was linear and reproducible between 5 and 30 nanomoles.

Isotachophoretic analysis of taurine in heart extract is shown in Fig. 1. Good resolution was obtained. A comparison of the determination of taurine in several

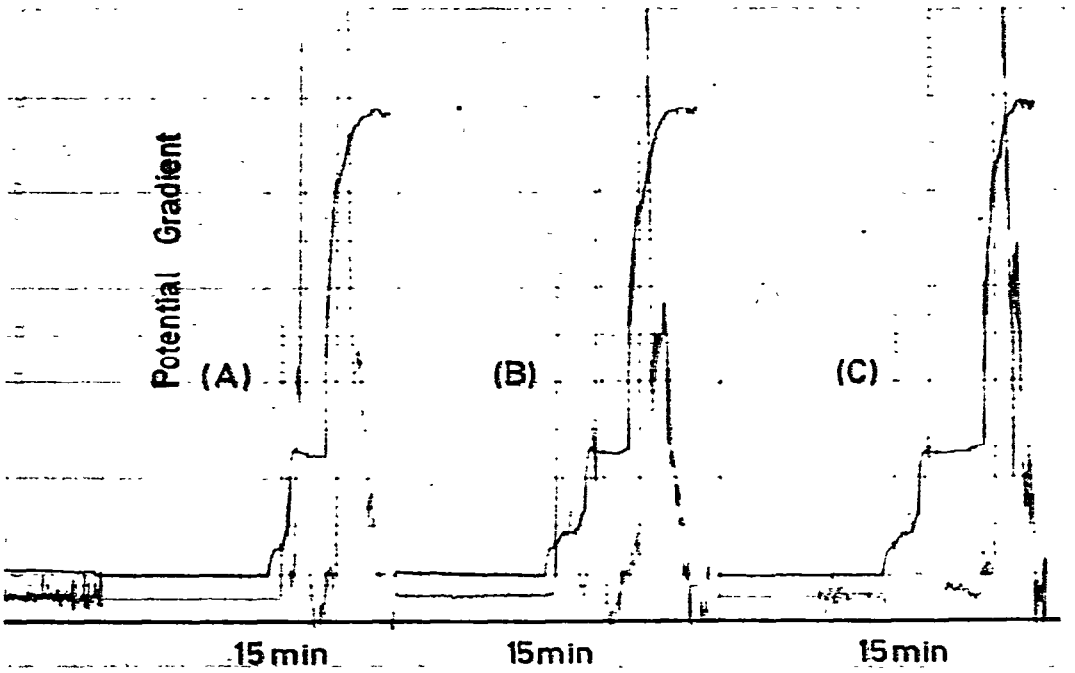

Fig. 1. Isotachophoresis of taurine: A, authentic taurine; B, heart extract; C, heart extract + taurine. The leading electrolyte was 0.01 *M* HCl and amidiol, pH 6.50. The terminator was 0.01 *M* γ-aminoburytic acid and $Ba(OH)_2$, pH 10.90. Migration current, 75 A; chart speed, 10 mm/min, temperature of thermostat, 20°C.

TABLE I

COMPARISON OF TAURINE CONTENTS IN VARIOUS ORGANS OF THE RAT DETERMINED BY ISOTACHOPHORETIC ANALYZER AND AMINO ACID ANALYZER

Organ	*Taurine contents (μmole/g wet weight)*	
	Isotachophoretic analyzer	*Amino acid analyzer*
Heart		
Ventricles	32.7 (27.9)	30.92 (27.14)
Auricles	16.9	15.74
Liver	9.6	9.53
Skeletal muscle	16.3	15.6
Serum	0.89	0.54

tissues and serum using isotachophoresis and the amino acid analyzer is shown in Table I. The two methods of analysis gave almost the same values. The contents of taurine in heart extracts could be analyzed directly without column chromatography. The results were in good agreement with the values obtained after separating taurine on a column.

The pretreatment required for determination of taurine using the isotachophoretic analyzer was more simple than that for the amino acid analyzer previously described, and this method is very useful for detecting taurine in several tissues.

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