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Note

Determination of taurine in foods and feeds using an amino acid analyser

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Taurine is an essential amino acid for cats as they are unable to synthesize it from methionine or cystine. However, also in the newborns of other species, including man, the level of taurine seems to be limited (for a summary, see, *e.g.*, ref. 1). Human milk contains a high concentration of taurine whereas cows' milk and formula diets based on milk products are very low in taurine[1,2].

The separation of taurine on an amino acid analyser, where it elutes near the void volume, can be difficult in certain foods such as some milk products that contain several other ninhydrin-positive compounds coinciding with taurine[2]. Moreover, the newer, accelerated and miniaturized systems, which additionally often exhibit a high relative void volume, fail to separate taurine from other compounds.

We have previously analysed the taurine content of diets for cats[2] without any difficulty using an older type of analyser, but experienced problems with a more modern system mainly when applied to milk and milk products. After introducing some modifications, which seem to us to be generally applicable and useful in similar instances, we achieved a clear separation of all compounds known at the moment possibly to interfere with taurine.

MATERIALS AND METHODS

Standard substances were obtained from Serva (Heidelberg, G.F.R.) and glycerophosphorylethanolamine (GPEA) from Sigma (Munich, G.F.R.). Human milk samples were obtained from the Clinical Hospital for Gynaecology and Obstetrics, University of Kiel, and cows' milk from several farms in Kiel area and from commercial products. The whey products and the baby food samples were commercial products from food manufacturing plants.

The amino acid analyser was a Liquimat III (Kontron Instruments, Munich, G.F.R.) working with a high-temperature coil and using a 160 × 4 mm column packed with the resin MCI Gel 10 F, particle size 7 μm (Mitsubishi, Japan). The flow-rate of the buffers was reduced from 20 to 12 ml/h and that of ninhydrin from 10 to 8 ml/h, which resulted in a reaction time in the coil of 3 min. With this system taurine produced the typical blue colour with ninhydrin at a relative intensity of 1.13 compared to equivalent amounts of norleucine (= 1.00). The colour production of urea was very low (< 0.1) at the reaction temperature of 117°C normally used but could be enhanced by increasing the reaction temperature (up to about 0.3 at 125°C). The void volume (volume from sample loop to resin surface) was 160 μl.

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The level of taurine in the foods was determined after extraction of the food samples with 0.1 *M* hydrochloric acid and deproteinization with sulphosalicylic acid, reaching a final concentration of about 3%. The deproteinized extracts were buffered with pH 1.8 sodium citrate buffer, then the samples were deep frozen for several days and then centrifuged again in order to remove the fine precipitate which cannot be removed from the fresh extracts. The sample volume injected by the automatic sample loading system was 100 μl.

RESULTS AND DISCUSSION

With the procedure (see Fig. 1a) commonly used for the determination of amino acids, we experienced problems with the separation of taurine from phosphoethanolamine (PEA) and urea. The other components tested in this study, namely cysteic acid, homocysteic acid, cysteinesulphinic acid and phosphoserine, caused no problems. Even glycerophosphorylethanolamine (GPEA), known often to co-elute with taurine[3,4], could be separated.

The problems with PEA and urea could be solved by reducing the column temperature from >40°C to 20°C and using an eluting buffer of pH 2.95 containing 0.12 *M* Na^+ (if the NH_3 trapping pre-column was omitted) or pH 2.66 (together if this pre-column was used).

Fig. 1 shows the amino acid analyser profiles for PEA, taurine and urea using different elution systems. The best separation could be achieved with column temperatures below 20°C but temperatures lower than 19°C caused too high back-pressures. In some special instances, as in the determination of taurine in several whey

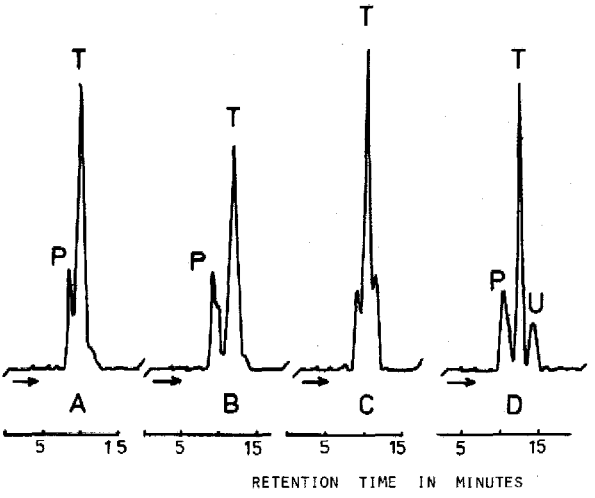

Fig. 1. Some amino acid analyser profiles demonstrating the separation of taurine from phosphoethanolamine (PEA) and urea. P = PEA; T = taurine; U = urea. In the standard profiles 2 nmol of PEA, 4 nmol of taurine and 20 nmol of urea were used. The coil temperature was 125°C. (A) Column temperature, 48.5°C; buffer, 0.16 *M* Na^+, pH 3.16; (B) as A, but column temperature 20°C; (C) column temperature, 48.5°C; buffer, 0.12 *M* Na^+, pH 2.95; (D) as C, but column temperature, 20°C. The retention times for taurine were A 9.9, B 12.1 and C 11.0 min; the retention times with sample D were 9.6 min for PEA, 12.2 min for taurine and 14.5 min for urea.

products, even lower temperatures together with a reduced flow-rate of the buffer may be recommended if high levels of urea and other interfering compounds crowd the taurine peak. As can be seen from Fig. 1, a decrease in temperature mainly improves the separation between taurine and PEA. The separation between taurine and urea could be achieved by the combined action of reducing the temperature and optimizing the characteristics of the buffer. Compared with other procedures (*e.g.*, refs. 4 and 5), our proposed procedure is mainly characterized by the low elution temperature. We consider that this modification should make it possible to separate taurine from other interfering substances even under unfavourable conditions and in delicate material such as whey products.

So far we have analysed 35 samples of human milk, 16 samples of cows' milk, 12 samples of dried whey or preparations from whey of different qualities and 24 samples of baby food using the described method. The average taurine values for these samples were 460 $\pm$ 220, 70 $\pm$ 30, 90 $\pm$ 40 and 38 $\pm$ 11 mg per kg dry matter.

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