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Note

Taurine levels in cat plasma

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Taurine is important in the elimination of bile[1]. The bile acids are conjugated with glycine and taurine in the liver, yielding water-soluble bile acids that can form salts with sodium and potassium. These glycocholate and taurocholate salts give rise to a detergent effect in the intestine that aids in the digestion of fats[2].

Taurine is not considered to be an essential amino acid for the well being of man, as it can be synthesized *in vivo* from cysteine or methionine[3]. But taurine is an essential amino acid for cats as they are unable to synthesize it from the other sulphur-containing amino acids. Exclusion of taurine from a cat's diet will result in total retina degeneration —blindness[4].

The taurine molecule is incapable of being included in the peptide chain of a protein because of its structure. It is "loosely attached" to proteins and, thus, can be easily lost, for example, as in the process of pellet preparation of cat dried-food, by being leached out.

The level of taurine in a cat diet can be ascertained by analysis of deproteinised cat plasma with an amino acid analyzer. However, it has been previously determined that estimates of taurine in vertebrate brain tissue can vary widely depending upon the methods used for isolating and quantitating the amino acid[5]. Such a wide variation in taurine levels has been found to occur when analysing plasma that had been deproteinized with 5-sulphosalicylic acid (SA). This reagent has proved to be unsatisfactory because a fine precipitate slowly forms when the "deproteinised" plasma is stored at 5°C or ambient temperature. Thus, this communication deals with protein precipitation from plasma using SA, and describes a simple procedure for conversion of an initial finely divided precipitate (that is held in suspension) into flakes that can be more effectively removed from the free amino acid solution when loading onto an amino acid analyzer ion-exchange column.

EXPERIMENTAL

A Beckman 120B amino acid analyzer was used for the taurine analyses and titanous chloride-reduced ninhydrin reagent was used for colour development[6]. The analyses were carried out with Durrum DC-1A cation-exchange resin (57 cm) and the amino acids were eluted with pH 3.25 sodium citrate buffer. Sulphosalicylic acid was obtained from Ajax Chemicals, Sydney, Australia.

The red cells in the drawn blood (5 ml) were spun down in a Epindorf bench

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centrifuge and a 0.4-ml aliquot of plasma was transferred to a clean plastic centrifuge tube. A 0.2-ml volume of the 12.5% SA solution was added. The samples were spun for 15 min and the supernatant transferred to a clean tube and spun again for 10 min. The cloudy supernatant was transferred to a thick-walled Pyrex glass tube (4 × 1 in.) and 0.2 ml of the ethanol–water (80:20) mixture added. The tube contents were evaporated to dryness on a rotary evaporator and to the residue was added 0.2 ml of

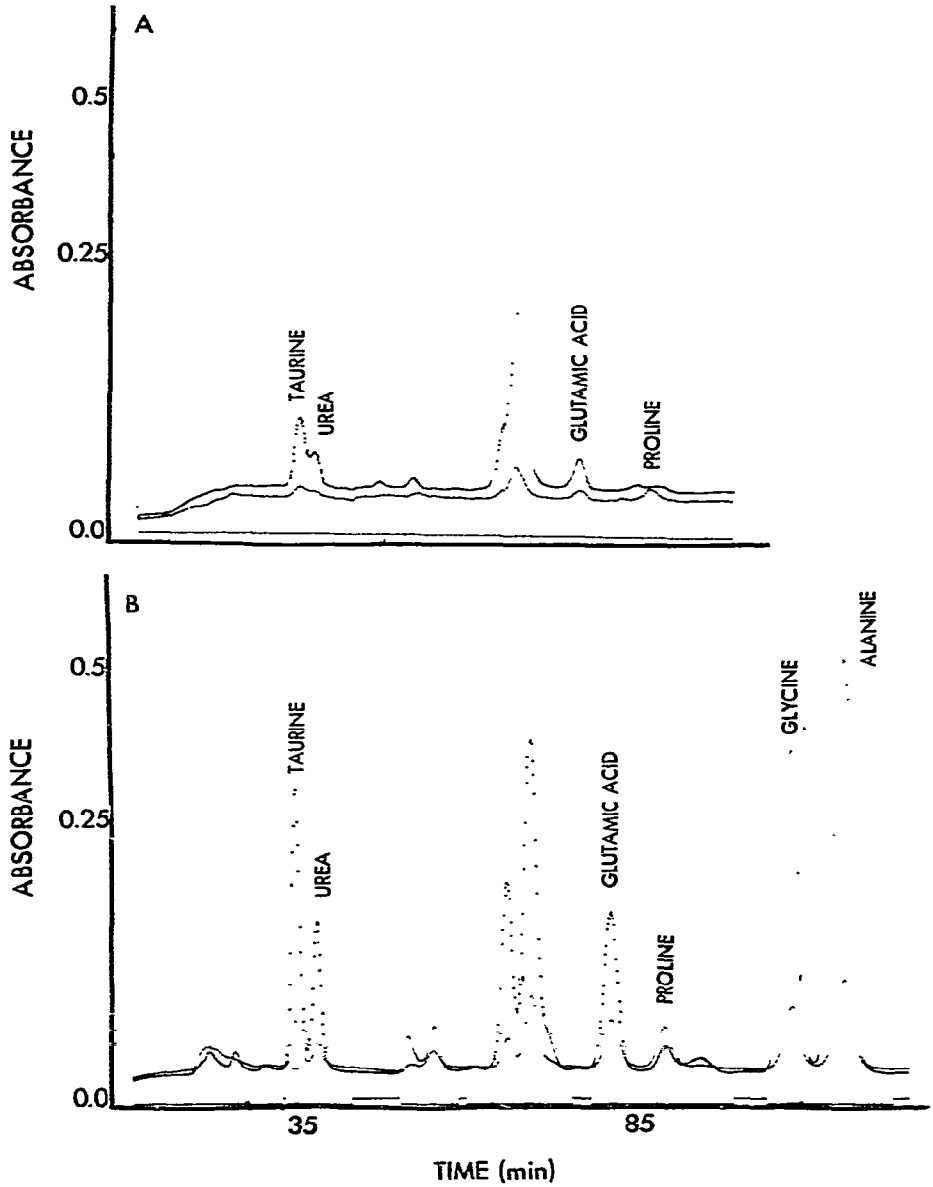

Fig. 1. Analyses of (A) a 0.2-ml aliquot of cat plasma, and (B) 0.4 ml of the same plasma. Sample A was treated with SA. Sample B had been treated with SA and 80% ethanol.

pH 2.2 sodium citrate buffer. All the contents of the tube were transferred to the amino acid column with a disposable pipette. A 0.2-ml wash with pH 3.25 sodium citrate buffer was also added to the column. A water pump vacuum line was placed inside the open end of the column close to the PTFE disc, capping the resin, and with a disposable pipette a jet of pH 3.25 buffer was forced against the PTFE disc and the suspension removed with the vacuum line. The analysis is then begun after filling the column void volume with pH 3.25 buffer.

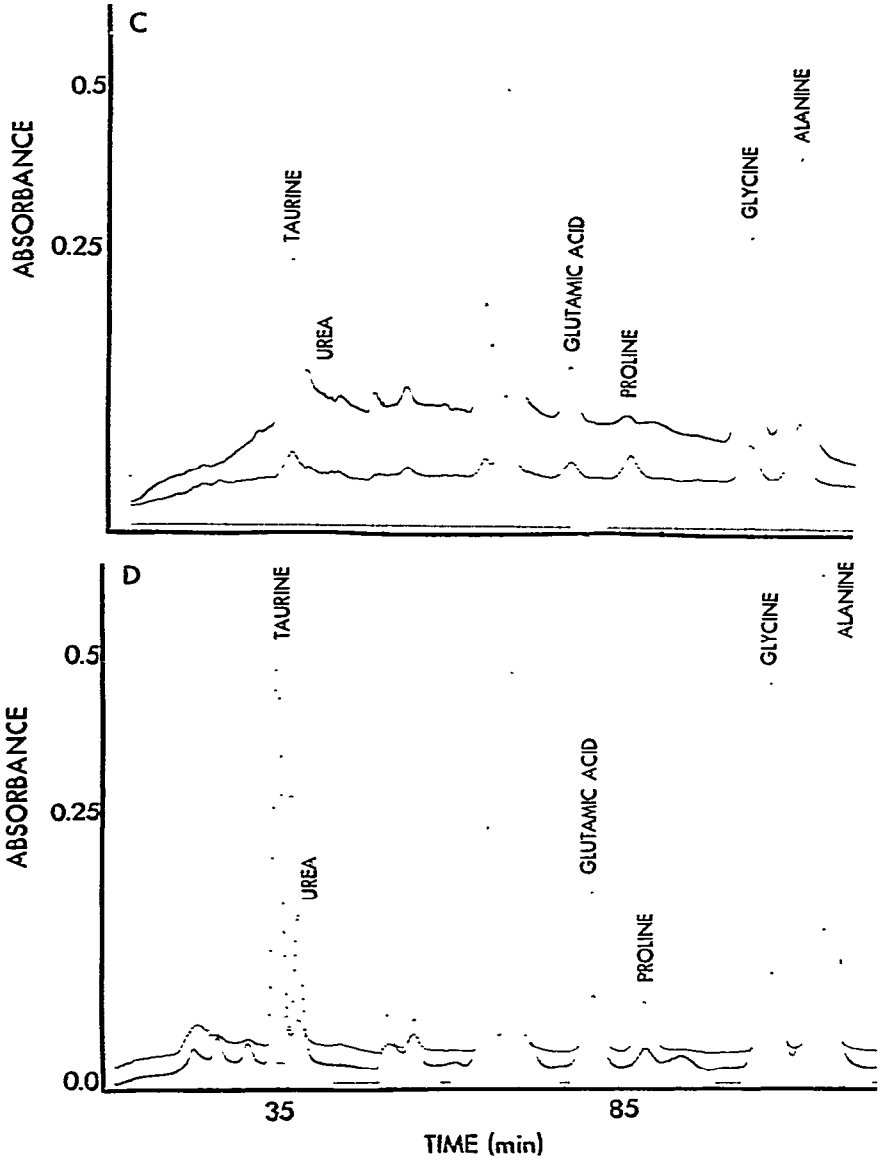

Fig. 2. Analyses of 0.4-ml aliquots of the same cat plasma. Sample C was treated with SA. Sample D had been treated with SA and 80% ethanol.

RESULTS AND DISCUSSION

The results of analysis of cat plasma to determine free taurine levels are shown in Figs. 1 and 2. The chromatograms labelled C and D were obtained with duplicate aliquots of plasma, but the sample in chromatogram C had not been treated with 80% ethanol. In chromatogram D the advantage of the 80% ethanol treatment is clearly demonstrated; the taurine peak is completely resolved from urea and is much larger, the baseline printout of the recorder is also much improved. Plasma from the same cat was used to obtain chromatograms A and B. A 0.2-ml volume of plasma had been used to obtain chromatogram A and a 0.4-ml volume to obtain chromatogram B. This experiment had been carried out to determine whether resolution of taurine and baseline printout could be improved by using less sample for the analysis. As can be seen, no advantage was gained by reducing the volume of plasma analysed.

The differences between SA alone and SA plus 80% ethanol-treated samples is not only demonstrated by the complete resolution of taurine and urea, but also throughout the chromatogram development. Further, in every analysis undertaken with identical aliquots of cat plasma the samples treated with SA and 80% ethanol provided higher levels of recovery of taurine and other amino acids than samples treated with SA only.

Chromatograms E, F and G in Fig. 3 show the reproducibility obtainable with triplicate aliquots of cat plasma that were deproteinised with SA and 80% ethanol. The peaks upon integration gave the equivalent of 63.0 nmoles, 63.0 nmoles and 62.8 nmoles for taurine in samples E, F and G respectively.

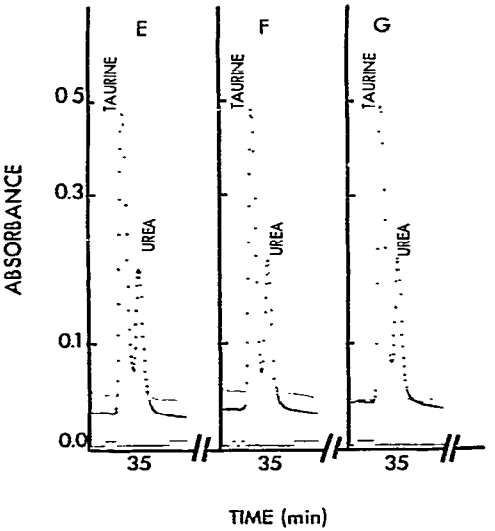

Fig. 3. Chromatograms E, F and G are triplicate analyses of cat plasma that had been treated with SA and 80% ethanol.

Deproteinisation of plasma using picric acid was not attempted. However, the reliability and quantitation accuracy obtained with the picric acid procedure is well documented[7,8]. The advantage of using SA followed by 80% ethanol deproteinisation over the picric acid method is one of convenience; it is quicker to carry out and does

away with the necessity of passing the sample down a Dowex 50 resin column to remove the yellow-coloured picric acid, before amino acid analysis.

As mentioned above, the domestic cat is very vulnerable to retina degeneration through the absence of taurine in its food. This condition can readily arise in cat breeding establishments, especially when the cat food is predominantly in the dry pellet form. Indeed, some plasma from domestic cats, when analysed, showed no taurine, and in each of these cases retina degeneration was detected[9].

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