

Occurrence of Phenylacetylglutamine in Cow's Milk

PHENYLACETYLGLUTAMINE is a normal constituent of human urine¹, but other mammals including the dog, cat, rat, monkey, sheep and horse do not excrete this compound². Phenylacetic acid administered orally to mammals results in the excretion of phenylacetyl glycine. The synthesis of phenylacetylglutamine by human tissue has been described by Moldave and Meister³.

During the course of an investigation on the non-protein-nitrogen fraction of cow's milk by ion-exchange chromatography⁴, a fraction was obtained in small quantities which was identified as phenylacetylglutamine. The occurrence of this compound in the milk of any mammal has not to our knowledge been reported previously. Evidence to substantiate the natural existence in cow's milk of phenylacetylglutamine is given here with some characteristics of the compound.

Authentic phenylacetylglutamine was prepared by the method of Thierfelder and Sherwin⁵. Paper chromatography was conducted on 8-in.² sheets of Whatman No. 1 paper using the ascending technique. Desalting of the fractions from the Moore-Stein column was accomplished following the method of Dreze *et al.*⁶. The unknown was isolated from a very small peak falling between urea and aspartic acid in the area normally occupied by methionine sulphoxide. The desalted unknown and authentic phenylacetylglutamine displayed the following properties: ninhydrin-negative on paper; failed to form an *N*-chloro derivative with chlorine gas⁷ or with *tert.*-butylhypochlorite⁸; reacted readily with ammoniacal silver nitrate on papergrams; migrated identically in six different solvent systems (the six solvent systems were: MeOH/H₂O/pyridine (80 : 20 : 4); *tert.*-BuOH/H₂O/HCOOH (69.5 : 29.5 : 1); 80 per cent EtOH; EtOAc/MeOH/H₂O (50 : 25 : 25); 80 per cent *n*-PrOH; 2-butanone/*tert.*-BuOH/H₂O : diethylamine (40 : 40 : 20 : 4)); in the solvent system 2-butanone/*tert.*-butanol/water/diethylamine (40 : 40 : 20 : 4), two spots were obtained both browning and showing high fluorescence when the paper was heated in an autoclave. Attempts to hydrolyse both samples in 5.7 *N* hydrochloric acid at 110° for 10 days gave no detectable glutamic acid. Partial hydrolysis was effected in 6 *N* sulphuric acid

at 180° C. for three days, the glutamic acid being isolated by adsorption on 'Amberlite IR-120 (H⁺)'.

Isolation of crude phenylacetylglutamine from cow's milk was also accomplished as follows: Six litres of fresh, raw whole milk were concentrated to about 2 litres *in vacuo*, treated with an equal volume of 20 per cent trichloroacetic acid and filtered after 1 hr. The clear filtrate was extracted continuously for 40 hr. with ethyl ether. The serum was adjusted to pH 3.1, then extracted continuously for 72 hr. with ethyl acetate. The latter was evaporated *in vacuo*, the residue taken up in 10 ml. water and shaken for 1 hr. with 0.5 gm. charcoal prepared by the Partridge method⁵. The charcoal was filtered, washed with water and the filtrate passed over 'Amberlite IR-120 (H⁺)'. Elution with 0.1 N hydrochloric acid gave crude phenylacetylglutamine having properties identical with the synthetic sample and with the fraction isolated from the Moore-Stein column. On the basis of the material isolated in this manner, it is estimated that milk contains at least 0.1 mgm. phenylacetylglutamine per litre.

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² Williams, R. T., *Detoxication Mechanisms* (John Wiley and Sons, Inc., New York, 1947).

³ Moldave, K., and Meister, A., *J. Biol. Chem.*, **229**, 463 (1957).

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⁶ Dreze, A., Moore, S., and Bigwood, E. J., *Analytica Chim Acta*, **11**, 554 (1954).

⁷ Bydon, H. N., and Smith, P. W. G., *Nature*, **169**, 922 (1952).

⁸ Schwartz, D. P., and Pallansch, M. J., *Anal. Chem.*, **30**, 219 (1958).

⁹ Partridge, S. M., *Biochem. J.*, **44**, 521 (1949).