PROTEIN HYDROLYSATES FOR THE MICRO-BIOLOGICAL ASSAY OF AMINO-ACIDS*

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THE circumstance that certain amino acids in a protein hydrolysate can be selectively and quantitatively removed or destroyed, offers the attractive possibility of employing such hydrolysates in the micro-biological assay of some amino acids. For tryptophane assay for example, Green and Black[1] have used an acid hydrolysate of casein; Lyman[2] has used H_2O_2 treated peptone for methionine assays. In the course of nutrition studies, protein hydrolysates have been widely employed from which a given essential amino acid is eliminated by suitable treatment. Wood, *et al.*[3] have used freshly prepared Raney's nickel for the removal of organic sulphur from sulphur containing amino acids from a protein hydrolysate. Ion-exchange resins have been used by Sperber,[4] and Cannan[5] for the removal of di-carboxylic and the basic amino acids. Lewis,[6] *et al.* have removed glutamic acid quantitatively from casein hydrolysate as pyrolidonecarboxylic acid.

Experimental

Acid hydrolysate of casein was prepared by the method of Snell and Wright[7] and alkali hydrolysate was prepared using barium hydroxide which is quantitatively removable as sulphate. Acid hydrolysates, after supplementing with cystine, methionine, and fortification with vitamins, purine bases, salts, sodium acetate and glucose, have been found useful for tryptophane assays. Similarly alkali hydrolysates fortified with methionine, and arginine or cystine, have been found suitable for the assay of cystine and arginine respectively.

The pre-treated hydrolysates, before actual use, were tested for their freedom from a given amino acid by chromatographing the liquid on paper using the capillary ascent test tube method developed by Rockland and Dunn[8] with *n*-butanal-acetic acid as developing solvent. What is shown to be absent by this test has been found to reach the microbiological standard of purity, as can be seen by Table I.

TABLE I

Treatment of casein	Amino acid removed	Chromatogram test	Micro-biological test
Acid hydrolysis	Tryptophane	Spot absent	Absent
Acid hydrolysis	Tryptophane	Spot absent	Absent
and H_2O_2	Methionine	Intensity of Valine-Methionine spot reduced to half	Absent
Alkali hydrolysis	Cystine arginine	Intensity and diameter of basic amino acid spot is less than control	Both absent

Media for the assay of tryptophane, methionine, cystine and arginine:—

	Tryptophane	Methionine	Arginine	Cystine
Basal medium 15 ml. each (composed of all vitamins, purine bases, salts and sugar) ..	+	+	+	+
Acid hydrolysate of casein ..	+	−	+	−
Alkali hydrolysate of casein ..	−	−	+	+
H_2O_2 treated acid hydrolysate of casein ..	−	+	−	−
Tryptophane ..	−	+	−	−
Methionine ..	+	−	+	+
Arginine ..	−	−	+	+
Cystine ..	+	+	+	−

Volume was made up to 50 ml. in each case, and the pH of the media adjusted to 7·2.

1·5 ml. of the double concentration medium was transferred to 4 ml. capacity pyrex tubes (4" × ½") and graded doses of tryptophane, methionine, cystine or arginine as the case may be were added and the volume in each tube made up to 3 ml. by adding the requisite quantity of distilled water. The tubes were steam sterilised for half an hour, cooled and inoculated with the thrice washed saline suspension of the test organisms. After 72 hours of

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incubation at 37° C. the entire quantity was titrated against N/20 NaOH. The results are graphically illustrated.

Amino acid	Organism	Range	Remarks
Tryptophane	*L. arabinosus*, NCTC 2161	0·0–2·0γ	Ideal
Methionine	*Leuconostoc mesenteroides* P–30 NCTC 2177	0·0-15·0γ	Ideal
Arginine	S–190, NCTC 2185	0·0–50 0γ	Ideal
Cystine	*L. Casei* ε, NCTC 2153	0·0–20·0γ	Ideal

Discussion

The pre-treated hydrolysate provides a simple, inexpensive and well-balanced mixture which can safely replace the usual medium compounded from individual

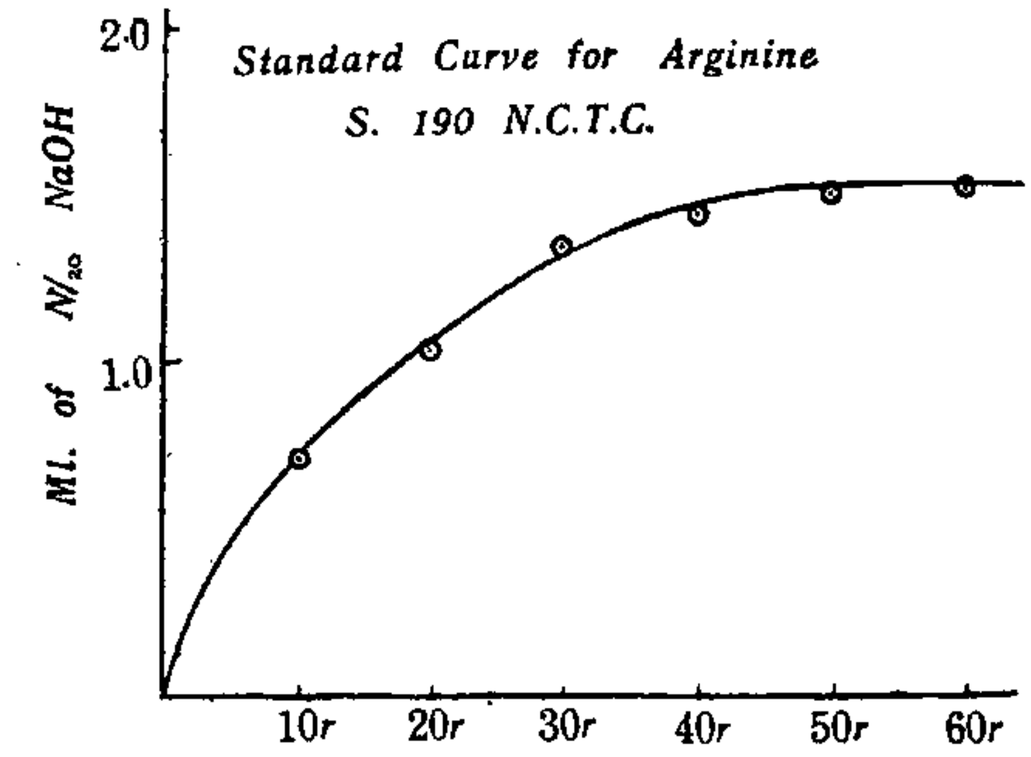

FIG. 1

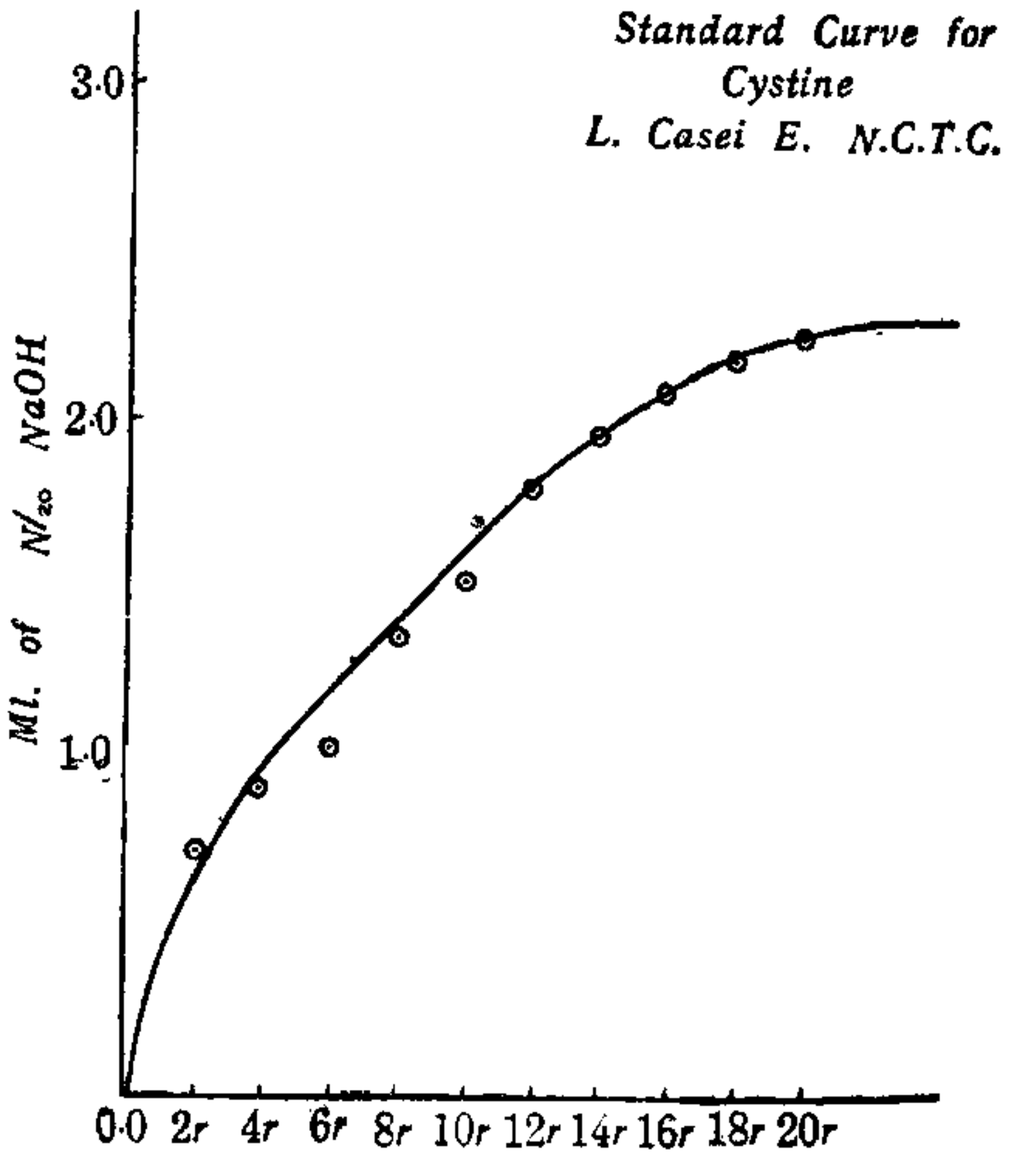

FIG. 2

amino acids. The use of expensive amino-acids which are now difficult to import can be mininised for most of the assays. These pretreated casein hydrolysates have been utilised for obtaining the standard curves for arginine, cystine, methionine and tryptophane (see Figs. 1-4) and are now

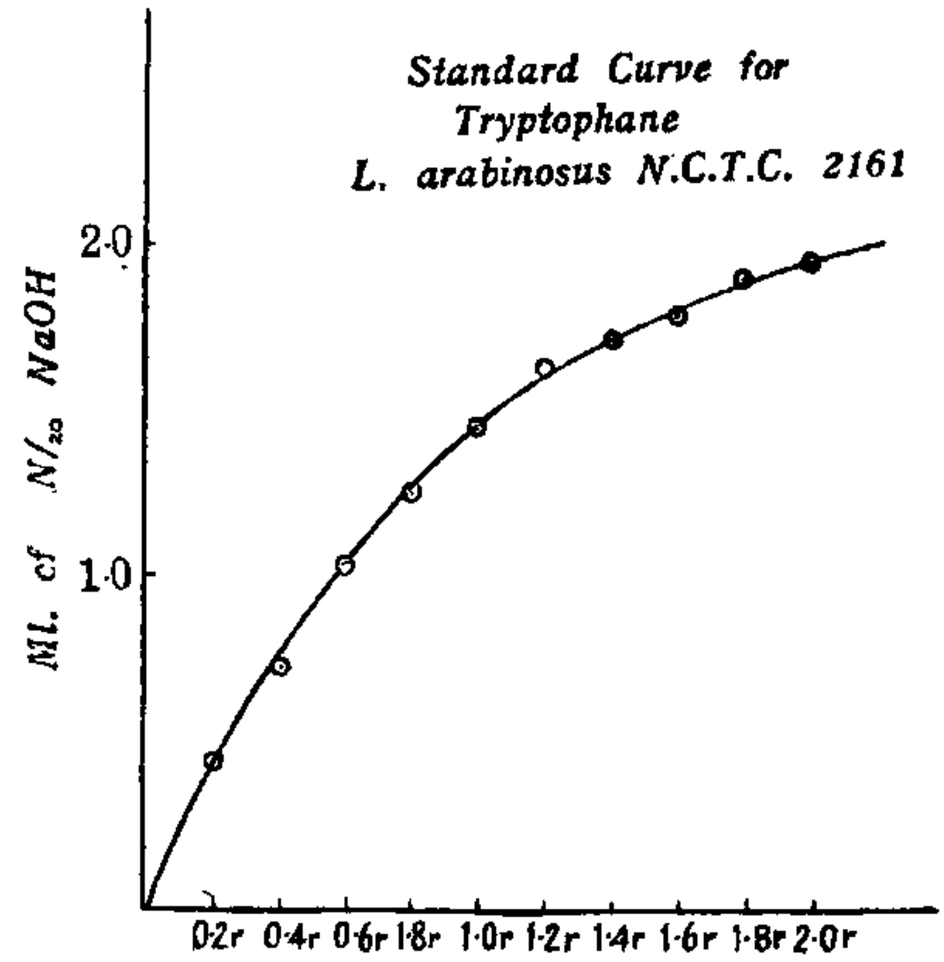

FIG. 3

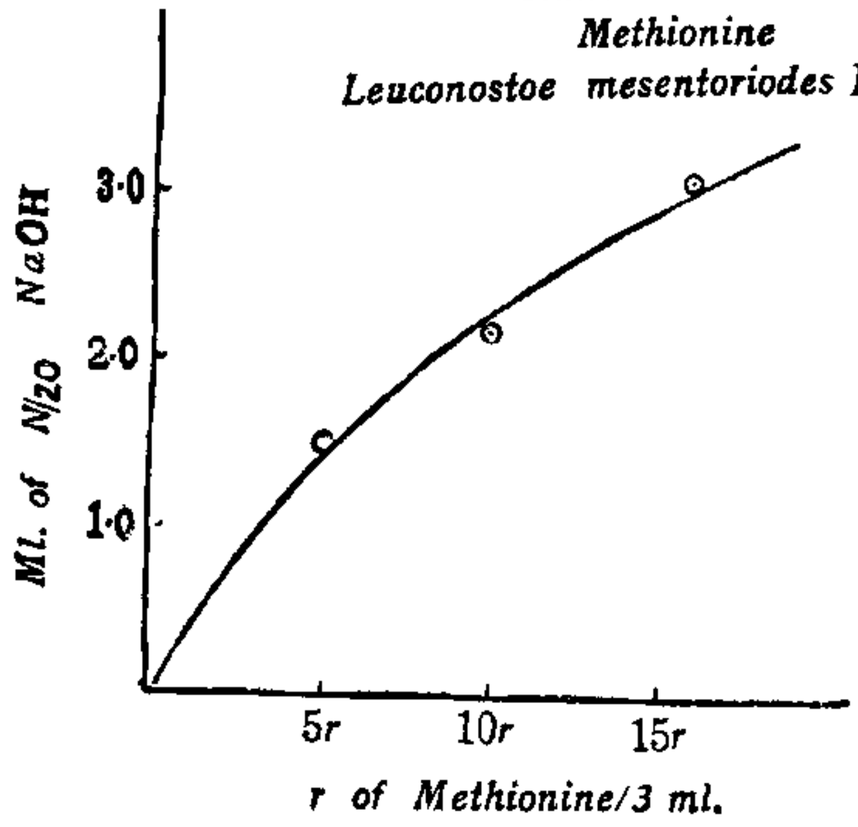

FIG. 4

being extensively employed in our laboratories for the routine assay of these amino acids in biological materials.

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1. Green, R. and Black, A., *Jour. Biol. Chem.*, 1944, **155**, 1. 2. Lyman, C., *et al.*, *Arch. Biochem.*, 1946, **10**, 427. 3. Wood, John L., *et al.*, *Federation Proceedings*, 1949, Part I, 266. 4. Sperber, E., *Jour. Biol. Chem.*, 1946, **166**, 75-78. 5. Cannen, R. K., *Ibid.*, 1944, **152**, 401. 6. Lewis, J. C., and Olcott, H. S., *Ibid.*, 1945, **157**, 265. 7 Snell, and Wright, *Ibid.*, 1941, **139**, 675. 8. Rockland, B., and Dunn, M. S., *Science*, 1948, **108**, 213.