A Colorimetric Procedure for the Determination of Aspartic Acid.* (17934)

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(Introduced by Arnold H. Maloney)
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Although there are many procedures in the literature for the determination of aspartic acid it now seems certain that classical isolation methods have yielded low results(1). Microbiological assays are specific for the L isomer only(1). Braunstein's(2) modification of Dakin's(3) method which is specific, accurate, and non-selective with respect to the D and L forms, is based on the stoichiometric conversion of aspartate to fumarate (2,3). Fumaric acid is then reduced to succinate and the latter is measured with succinic dehydrogenase. The present report describes a procedure which eliminates this reduction and enzymatic assay and analyzes colorimetrically the fumaric acid produced from aspartic acid with dimethyl sulfate. The determination is applicable to amino acid mixtures and hydrolysates when the amount of aspartic acid measured exceeds 0.5 mg. Asparagine interferes with this analysis as with the enzymatic method.

Procedure. One ml of aqueous solution containing from 0.6 to 1.4 mg of aspartic acid was pipetted into a 20 ml beaker and made alkaline with sodium hydroxide. Alternately methyl sulfate (Daigger and Company, Chicago, Ill., Highest Purity) and 33% sodium hydroxide were added dropwise to the mixture over a one hour period. The final volume of methyl sulfate and sodium hydroxide added was 0.25 ml each. The solution at no time became acid. Between the additions the beaker was agitated on the Dubnoff metabolic shaker. It was placed in a refrigerator overnight and on removal the solution was adjusted with 10 N sulfuric acid to approximately pH 2. The acid contents were mixed with 1 g of dry silica, prepared as described by Marshall, Orten and

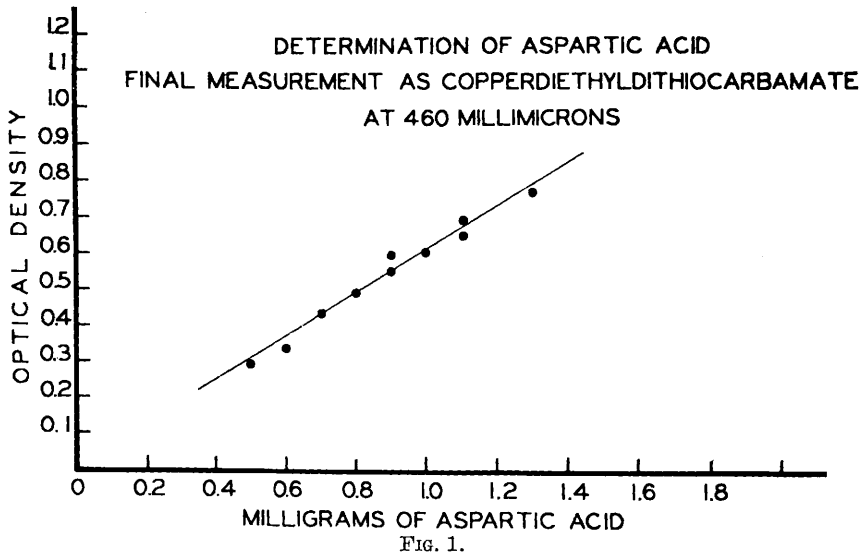

FIG. 1.

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TABLE I.
Analysis of Aspartic Acid in Protein Hydrolysates.

Protein	Nitrogen, %	Aspartic acid, %	
		Observed	Literature
Crystalline lysozyme	16.90‖	10.60†	10.2 (6)
Crystalline bovine serum albumin*	15.75§	9.92‡	10.25 (7, 8) 10.9 (9) 11.1 (10)

* Kindly supplied by Dr. Max S. Dunn.
† Average of 3 determinations.
‡ " " 2 "
§ Corrected for moisture and ash (moisture, 1.84%; ash, 0.55%).
‖ Moisture and ash not determined.

TABLE II.
Recovery of Aspartic Acid from Amino Acid Mixtures.

Determination No.	Synthetic mixture*			Casein hydrolysate†		
	Added, mg	Found, mg	Recovery, %	Added, mg	Found, mg	Recovery, %
1	.99	.89	90	0	0.67	
2	.99	.96	97	.93	1.59	99
3	.99	.94	95	.93	1.66	107
4				.47	1.16	104
Avg			94			103

* Per 100 ml of solution the mixture was composed of DL-tyrosine 10.3, tryptophane 2.2, cystine 1.4, lysine 2.1, arginine 2.7, glutamic acid 18.6 mg.
† The casein contained 13.4% N and 6.75% of aspartic acid.

Smith(4), suspended in 15 ml of 25% (volume/volume) butanol in chloroform and filtered through paper. After the residues were washed with 20 ml of the butanol-chloroform mixture the filtrate and combined washings were dried in a current of air at room temperature. The dry residue of fumaric acid was dissolved in 1 ml of warm water (70°) and transferred to a centrifuge tube with two 0.25 ml washings. The fumaric acid was precipitated as the copper-pyridyl complex(5) and the copper thus combined was determined colorimetrically(5). The calibration curve is shown in Fig. 1. The Beckman, Model DU, was employed for the photometric measurements.

Hydrolysates. Crystalline bovine serum albumin and crystalline lysozyme were refluxed for 20 hours with 10 times their weight of 6 N hydrochloric acid. When less than 0.8 mg of aspartic acid was assayed, the formation of the copper-pyridyl fumarate, in the final measurement, was favored by scratching the inner surface of the centrifuge tube with a small glass rod. Application of the procedure to hydrolysates provided the data in Table I.

Recovery studies. The analysis of the amino acid in mixtures is shown in Table II. Two amino acid preparations were used. One was synthetic; the other was a hydrolysate of casein. In 8 other samples, each containing 0.97 mg of aspartic acid by weight, the average amount found was 0.93 mg, the standard deviation ±0.06 mg.

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Discussion. The foregoing procedure is recommended for the determination of aspartic acid in protein hydrolysates. Biological fluids in general cannot be measured because of possible interference by fumaric acid. Although the use of the chromatographic determination for fumaric acid(4) would improve the sensitivity, a prior analysis of fumaric acid in the sample would decrease the convenience of the method. Obviously, the precision, specificity and sensitivity of the final measurement can be no greater than that reported for the conversion(3) and the colorimetric procedure for fumarate(5).

Summary. A procedure is described for the determination of aspartic acid in quantities of 0.5 mg or above in the sample. The amino acid is converted to fumaric acid and this is measured colorimetrically.

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Methylene Blue, 2,4-Dinitrophenol, and Oxygen Uptake of Intact and Homogenized Embryos.* (17935)

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That M.B. and 2,4-D.N.P. in suitable concentrations stimulate or augment the oxygen consumption of intact cells and tissues of a variety of biological forms seems well established(1,2,3). The exact mechanisms involved in such reactions, however, do not seem to be so well understood and many ideas have been advanced in attempting explanations of such phenomena. More recent investigators of the action of these chemicals upon the oxygen uptake of intact and homogenized cells emphasize the importance of factors associated with cell structure in conditioning the results(1,4). Marked effects upon intact cells have been pointed out while quite opposite and at times contrary results are described for homogenates and intracellular constituents of the same materials(1). Additions of various substrates to homogenates in certain instances seem to condition or at least influence the nature of the response(1,4).

Since much of the available data seems to have been obtained for mammalian tissues requiring the addition of substrates for the reaction it was thought desirable to extend these observations to the embryonic cells of invertebrates which normally do not require the addition of substrates for such studies. The intact embryo of the grasshopper, *Melanoplus differentialis,* which has proven of value for various cellular problems, has been used in the present investigation(2,3). Homogenates as well as intracellular constituents (nuclei, mitochondria, microsomes) prepared by fractional centrifugation techniques have also been employed. The suspension medium used throughout all experiments was .9% NaCl, buffered to pH 6.8 by .006M phosphates. No additional substrates were added so that oxygen uptake, as measured by standard Warburg manometers, represents that necessary for endogenous respiration. Respiration flasks, 5 ml capacity, were used and all experiments were conducted at 25°C. One hundred embryos per cc of suspension medium or their equivalent were used throughout. Homogenates were prepared by means of an all glass Potter-Elvehjem homogenizer (5). Nuclei, mitochondria and microsomes

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