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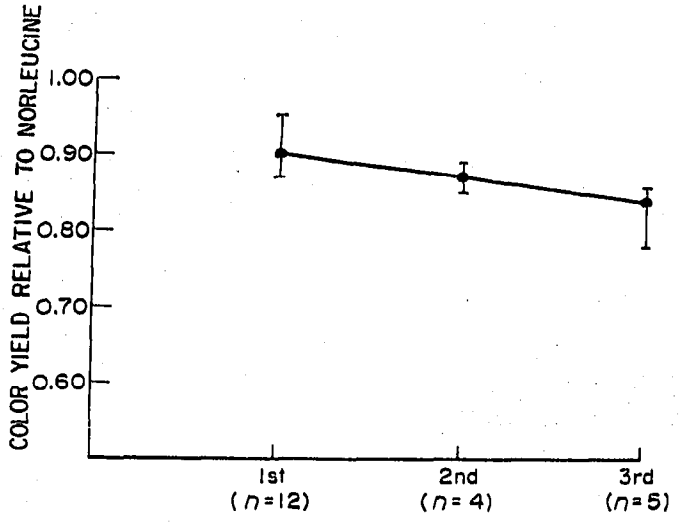
The progressive loss of aspartic and glutamic acids using a multiple column analytical system

Our laboratory is engaged in the amino acid analysis of proteins isolated from arctic mammals as part of a larger program involving genetic differences and speciation at the molecular level. We are using an ion exchange chromatography method based on the PIEZ gradient system[1] in which a battery of five 6 mm columns is programmed by a sequential switching system.

In the normal daily routine, samples are applied to three or four columns simultaneously. Except for the sample on the initial column which is immediately analyzed, the samples remain adsorbed at the tops of their columns for multiples of



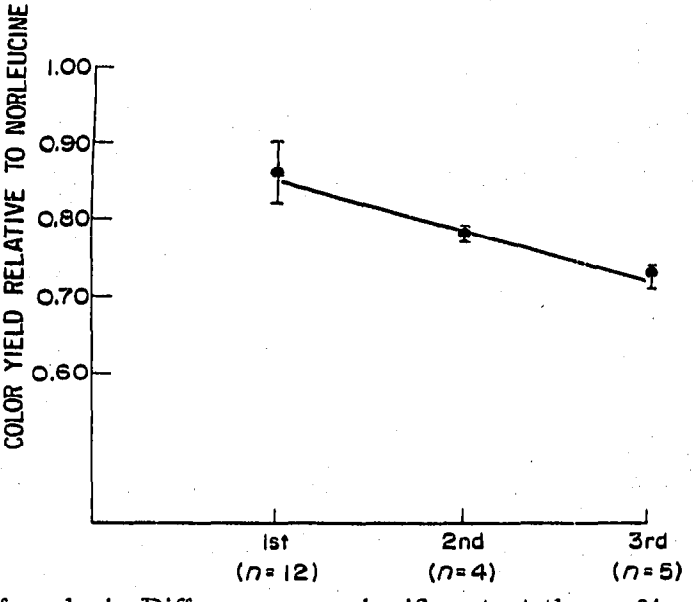


Fig. 1. Loss of aspartic acid on the basis of order of analysis. Differences are significant at the 95% level ("t" test).

Fig. 2. Loss of glutamic acid on the basis of order of analysis. Differences are significant at the 95% level ("t" test).

six hours before elution begins and are thus exposed to a pH of 2.75, a temperature of 60° and a methanol containing (10%) solvent system[2] for six to eighteen hours.

The values obtained, when compared or grouped on the basis of operational sequence, indicated no significant differences for the majority of the amino acids, but did indicate a progressive decrease in recovery of both glutamic and aspartic acids. Fig. 1 shows the results obtained for the latter.

Fig. 2 shows a similar plot obtained for glutamic acid. Consequently we have incorporated a correction routine into our data handling procedures. The mean values (Table I) are used to correct each analysis on the basis of operational sequence. These factors are included in a computer program which applies the correction on the basis of a sequence number given the analysis.

TABLE I

MEAN RECOVERY OF ASPARTIC AND GLUTAMIC ACID ON THE BASIS OF ORDER OF ANALYSIS RELATIVE TO NORLEUCINE

Acid	*1st* ($n = 12$)	*2nd* ($n = 4$)	*3rd* ($n = 5$)
Aspartic	0.90	0.87	0.84
Glutamic	0.86	0.78	0.73

Differences observed are significant at the 95 % confidence level.

To date, we have mostly negative information on the products formed. We have not found any positive correlation with any other peak elsewhere in the chromatogram.

It has been suggested that the color loss we have observed may be due to esterification[3]. In the case of aspartic acid the observed loss may very well be due to color yield differences of the methyl esters. If simple esterification is not the answer, another possibility is the conversion of the aspartic acid to fumaric or maleic acids. The ammonia released by this reaction might not be detected by our system. The large loss of glutamic acid is best explained by its conversion to ninhydrin negative pyrrolidone carboxylic acid[4]. Work to establish the degradation products is now in progress.

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Column separation of phosphatides, acyl carnitines and carnitine

The separation and isolation of carnitine derivatives has been reported[1–6]. Although individual carnitine derivatives may be isolated by these procedures, it is often desirable to separate and isolate the phosphatides, acyl carnitines and carnitine by a single procedure. This is particularly true if the starting material is insect tissue. In these organisms when radioactive carnitine is used as a substrate, the products