TABLE I

RECOVERY OF AMMONIA IN THE PRESENCE OF SUBSTRATE AND PRODUCT

Known amounts of ammonia, α-N-benzoyl-L-lysinamide (5 μmoles) and α-N-benzoyl-lysine (5 μmoles) were incubated in 2 ml of 0.01 *M* TES, pH 7.5, containing 0.05 *M* calcium chloride and 0.1 *M* sodium chloride. One milliliter of 20% sulfosalicylic acid was added to each mixture at the time indicated.

Time incubated (min)	*Ammonia added (μmoles)*	*Ammonia recovered (μmoles)*
10	0.30	0.29
20	0.60	0.61
30	0.90	0.93

Table I were obtained from a single chromatogram, they also show that the chromatographic method itself is applicable to the determination of ammonia in the presence of these other substances.

It should be noted that when several samples are successively deposited on a column as in the method described here, the components which are not retarded by the resin emerge with the void volume of effluent of the column shortly after each sample application and have therefore traversed the ammonia peaks of the previously applied samples on their way down the column. This apparently has no effect on the peaks. The components of the last peak applied, however, must emerge before the first ammonia peak to preclude interference with the colour development of the peak. With the Aminex A-5 system described here, the first ammonia peak emerges at 71 min, therefore up to five samples of ammonia, deposited on the column at 10-min intervals, could be determined on one chromatogram. If some component in the mixture other than ammonia is also retarted by the resin, not so many samples can be successfully

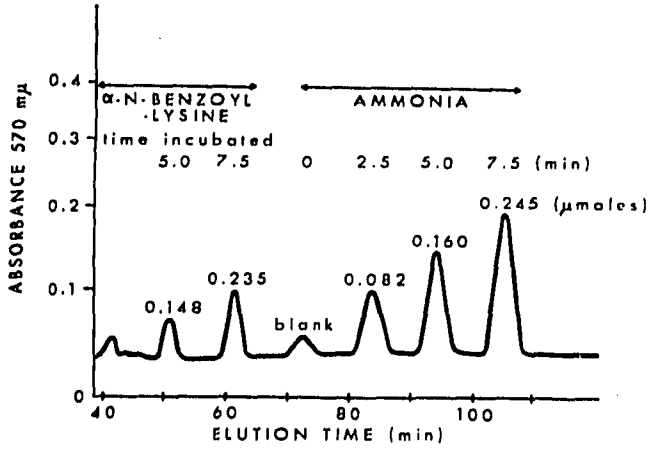

Fig. 3. Chromatography showing the hydrolysis of α-N-benzoyl-L-lysinamide by trypsin at pH 7.5 and 25°. Conditions as in Fig. 1. Trypsin solution was added to equal volumes of 0.02 *M* TES containing 0.1 *M* calcium chloride, 0.2 *M* sodium chloride, and α-*N*-benzoyl-L-lysinamide. Reactions were terminated at the times indicated by the addition of half a volume of 20% sulfosalicylic acid, and the mixtures centrifuged. An aliquot of each supernatant solution was placed on the column at 10-min intervals.

determined on one chromatogram. Such was the case encountered in our kinetic studies when ammonia was determined in the presence of α-N-benzoyl-lysine. The chromatogram from a typical kinetic experiment is represented in Fig. 3. It is seen that four samples could be determined, the first ammonia peak to emerge being preceded by the α-N-benzoyl-lysine from the last sample deposited on the column by only 11 min. This circumstance, however, proved fortuitous since the α-N-benzoyl-lysine was actually the other product of the reaction and it could be determined simultaneously with the ammonia on the same chromatogram. It is seen in Fig. 3 that very good agreement was obtained between the amounts of ammonia and α-N-benzoyl-lysine liberated by the enzymatic reaction after 5 and 7.5 min, respectively. This confirmed the reliability of the chromatographic method and moreover showed that ammonia assay was a reliable criterion as a measure of substrate hydrolysis.

The method described here has been used successfully for studying the kinetics of the trypsin-catalyzed hydrolysis of the α-N-benzoyl amides of ε-N-methyl-L-lysine and DL-homolysine[15], and is presently being used for studying the kinetics of the chymotrypsin-catalyzed hydrolysis of L-phenylalanine esters at basic pH values. For the latter, five samples of phenylalanine are analyzed as a set on one chromatogram using 0.2 *N* sodium citrate, pH 3.80, as the eluent. When the samples are placed on the column at 10-min intervals, the first peak emerges after 64 min, with the remainder following at about 5-min intervals. A similar result is obtained for the analysis of valine samples when the pH 3.28 buffer is used. The method can therefore probably be used for the determination of any amino acid once a buffer of appropriate pH has been found.

We acknowledge that the idea to develop this technique came from the work of JURASEK AND WHITAKER, who determined ammonia in sets of five samples with a single 6 to 7-h chromatogram of an amino acid analyzer after first having separated ammonia from protein using Conway cells[18].

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