10^{-3} min^{-1} respectively. Thus after five half-lives for the reactions in which compounds (I) and (II) are destroyed only about 45% of the theoretical amount of aspartic acid had been released in each case. Free L-aspartic acid was found to be stable under the conditions employed, both in the presence and the absence of free N-acetyl-D-glucosamine.

In 0.05M-sodium hydroxide at 100°C the first-order rate constants for the destruction of compound (I) and for the formation of aspartic acid from it were 3.1×10^{-3} and $0.87 \times 10^{-3} \text{ min}^{-1}$ respectively. These data and those obtained in 0.2M-sodium hydroxide may suggest that the reaction whereby compound (I) is destroyed without formation of aspartic acid is intramolecular. N-Deacetylated compound (I) is unlikely to be the major product in which the aspartic acid that is not released is incorporated, since acid hydrolysis of the alkali-treated material does not release aspartic acid except from un-degraded compound (I).

Compound (I) was destroyed at rates of $21 \times 10^{-3} \text{ min}^{-1}$ and $11 \times 10^{-3} \text{ min}^{-1}$ at 100°C in 0.2M- and 0.05M-sodium hydroxide respectively in the presence of 1M-sodium borohydride. Approx. 60 and 50% of compound (I) respectively were degraded to give aspartic acid with, in each case, similar amounts of reduced sugars. Substances with the chromatographic mobilities of asparagine and of homoserine are formed in the alkaline reductive cleavage of compound (I). The identities of these amino acids were confirmed from thin layer chromatographic studies of their dansyl derivatives.

Schiffman, G., Kabat, E. A. & Thompson, W. (1964). *Biochemistry, Easton*, 3, 113.

Tanaka, K., Bertolini, M. & Pigman, W. (1964). *Biochem. biophys. Res. Commun.* 16, 404.

The Determination of Methionine by Gas-Liquid Chromatography

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Under certain conditions peptide-bound methionine is sensitive to oxidation to the sulphoxide. Such oxidations may be intentional and specifically directed to establish the function of individual methionine residues in enzyme activity (Neumann, Moore & Stein, 1962), or they may be inadvertently incurred during some protein isolation procedures (Grant & Reid, 1968). A rapid method has been developed to monitor the extent of oxidation of methionine residues in proteins. It is based on the observation that, whereas the first stage of the modified Witkop reaction of cyanogen bromide with proteins is quantitative for methionine (Schroeder, Shelton & Shelton, 1969; Inglis &

Edman, 1970), yielding methyl thiocyanate, methionine sulphoxide does not react.

The protein was dispersed in concentrated formic acid before overnight reaction in 70% (v/v) formic acid with twice its weight of cyanogen bromide. 4-Methylcyclohexanone was added as an internal standard before the reaction mixture was adjusted to a final concentration of 30% (v/v) formic acid. Methyl thiocyanate was determined by g.l.c. and flame ionization in a Hewlett-Packard model 5750B analyser. The reaction of cyanogen bromide with free methionine took appreciably longer than that with the peptide-bound form, but this too was completed during the overnight incubation.

The method was applied to the measurement of the methionine content of lysozyme, ovalbumin, bovine serum albumin, β -lactoglobulin and two samples of casein. Results were compared with those obtained by the Moore & Stein analysis for methionine sulphone after performic acid oxidation (Moore, 1963). The two methods mostly agreed well, but the g.l.c. value for lysozyme was 7% low and that for β -lactoglobulin 7% high. Samples of casein oxidized by hydrogen peroxide (Ellinger & Palmer, 1969) were also examined. Their methionine sulphoxide content determined after alkaline hydrolysis (Neumann *et al.* 1962) corresponded to the loss of methionine as measured by the g.l.c. method.

Various reducing agents are being examined for their effectiveness in the conversion of the sulphoxide into methionine.

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Ellinger, G. M. & Palmer, R. (1969). *Proc. Nutr. Soc.* 28, 42A.

Grant, P. T. & Reid, K. B. M. (1968). *Biochem. J.* 106, 531.

Inglis, A. S. & Edman, P. (1970). *Analyt. Biochem.* 37, 73.

Moore, S. (1963). *J. biol. Chem.* 238, 235.

Neumann, N. P., Moore, S. & Stein, W. H. (1962). *Biochemistry, Easton*, 1, 68.

Schroeder, W. A., Shelton, J. B. & Shelton, J. R. (1969). *Archs Biochem. Biophys.* 130, 551.

Conjugates of Phenylacetic Acid with Taurine and other Amino Acids in Various Species

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Phenylacetic acid and certain other arylacetic acids are excreted in man and certain monkeys as glutamine conjugates, whereas in lower animals they form glycine conjugates (see Williams, 1967). An examination of the fate of [^{14}C]phenylacetic acid