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 undegraded 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.

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The Determination of Methionine by Gas-Liquid Chromatography

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Under certain conditions peptide-bound methionine is sensitive to oxidation to the sulfoxide. 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 sulfoxide 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 sulfoxide 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 sulfoxide into methionine.

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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

in various species has now shown that phenylacetic acid can form conjugates with glutamine, glycine, ornithine, taurine and glucuronic acid, the nature of the conjugates formed being dependent on the species. The taurine conjugate of phenylacetic acid is reported for the first time.

Phenacetylglutamine was found to be the only conjugate in the urine of man and the Old World monkeys, rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*M. fascicularis*), green monkey (*Cercopithecus aethiops*), drill (*Mandrillus leucophaeus*), white-collared mangabey (*Cercocebus torquatus*) and baboon (*Papio cynocephalus*). Both phenacetylglutamine and phenacetylglutamine were formed in the New World monkeys, squirrel monkey (*Saimiri sciureus*) and capuchin monkeys (*Cebus albifrons* and *Cebus nigrivittatus*). Phenacetylglutamine but no phenacetylglutamine was excreted by the lower primates slow loris (*Nycticebus coucang*) and bushbaby (*Galago senegalensis*) and by the following non-primates: rat, rabbit, guinea pig, mouse, hamster, cat, dog, ferret and vampire bat.

The hen was found to excrete mainly N^2N^5 -diphenacetylornithine (phenacetornithuric acid), together with smaller amounts of phenacetylglutamine. The pigeon, however, excreted, not diphenacetylornithine, but two other amino acid conjugates, one of which was readily identified as phenacetylglutamine, but the other did not correspond to the any of the known conjugates of phenylacetic acid. It was eventually identified, by using the mass spectrometer and amino acid analyser, as phenacetyltaurine.

An unknown conjugate had been detected in the urine of several other species, and from its R_f value on radiochromatograms this also appears to be phenacetyltaurine. It has been found in substantial amounts in the urine of the slow loris, bushbaby and ferret and in small amounts in the urine of the dog.

It is noteworthy that, as far as the glutamine and glycine conjugations of phenylacetic acid in primates are concerned, only glutamine conjugation occurs in man and the Old World monkeys, both glutamine and glycine conjugation occur in New World monkeys and only glycine conjugation occurs in the lemurs. A similar distribution of conjugation in primates has been found for indol-3-ylacetic acid (M. E. Evans, R. L. Smith & R. T. Williams, unpublished work; see Williams, 1971).

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The Biliary Excretion of Amphetamine and Methamphetamine in the Rat

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Both amphetamine and methamphetamine are excreted by the rat mainly in the urine (Dring, Smith & Williams, 1970; Caldwell, Dring & Williams, 1971), the major metabolic reaction being aromatic hydroxylation. The biliary excretion of these drugs in the rat has now been examined and, although the faecal excretion of these drugs is low (4-5% of the dose for amphetamine and 2% for methamphetamine), there is an appreciable biliary excretion of the metabolites of these drugs.

[^{14}C]Amphetamine sulphate (7.5 mg/kg) and [^{14}C]methamphetamine hydrochloride (7.5 mg/kg) were administered intraperitoneally in water to bile-duct-cannulated rats (Abou-El-Makarem, Millburn, Smith & Williams, 1967). The bile and urine were collected for 24 h and analysed for the ^{14}C -labelled metabolites by paper chromatography, radiochromatogram scanning and scintillation counting. In the case of amphetamine 69 and 16% of the dose of ^{14}C were excreted in the urine and bile respectively in 24 h, whereas with methamphetamine the values were 54 and 18%. The metabolites of amphetamine in the urine and bile respectively were: amphetamine, 24 and 1%; total 4-hydroxyamphetamine, 38 and 15%; hippuric acid, 6 and 0.1% of the dose. Similarly the metabolites of methamphetamine were: methamphetamine, 16 and 0.5%; amphetamine, 22 and 2%; total 4-hydroxymethamphetamine, 6 and 1%; total 4-hydroxyamphetamine, 4 and 1%; total 4-hydroxynorephedrine, 2 and 13% of the dose.

The main metabolite of amphetamine in bile was 4-hydroxyamphetamine glucuronide (12% of the dose) and that of methamphetamine was 4-hydroxynorephedrine glucuronide (12%). These two conjugates could be expected to be excreted in the bile in significant amounts, since their molecular weights (327 and 343 respectively) are in the lower range suggested by Millburn, Smith & Williams (1967) for extensive biliary excretion in the rat. The low faecal excretion and appreciable biliary excretion of certain metabolites of these drugs show that they can undergo an enterohepatic circulation in the rat. This suggests that on repeated administration of methamphetamine in the rat an accumulation in the body of 4-hydroxynorephedrine, which has been postulated as a false transmitter at nerve endings, might occur.

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