is also a reliable means of molecular-weight determinations.

A column (98cm $\times$ 2.5cm) packed with Sephadex G-200 (Pharmacia, Uppsala, Sweden) was used for these studies. All separations were carried out at 4°C. Calibration of the gel bed was with proteins of known molecular weight, and the void volume, V_0 , was determined with Blue Dextran. Calibration standards (15–20mg) dissolved in 3–4ml of 0.2M-KCl and 0.02% NaN_3 were applied individually to the column, equilibrated with same solution, by ascending flow at 10ml/h.

Aqueous extracts of acetone-dried muscle protein powders (Barany *et al.*, 1957) from dogfish, trout, chicken and rabbit were used as sources of actin. To maintain actin in the monomeric form (G-actin), the column was equilibrated with 0.5mM-ATP in 2.5mM-tris buffer, pH 8.1, plus 0.02% NaN_3 . Up to 20ml of 5mg/ml muscle extracts was applied to the column as described. Column effluents were collected in 5ml (calibration standards) or 10ml (muscle extracts) fractions.

Proteins were measured either by light-extinction at appropriate wavelengths or by the method of Lowry *et al.* (1951). Muscle extracts from each species separated into several peaks showing similar distribution and elution to those found by Adelstein *et al.* (1963) for the rabbit and Weihing & Korn (1971) for *Acanthamoeba*.

The location of the G-actin peak was established by its development of flow birefringence in 0.1M-KCl–10mM-MgCl₂. The purity of the actin was determined from gel electrophoresis, amino acid analysis and sedimentation analysis.

Our estimates of the molecular weight of rabbit actin (i.e. about 45000) do not differ significantly from other findings, and show that there is virtually no difference in this respect between the species studied.

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Sequence Studies on the Milk Protein α -Lactalbumin by using Mass Spectrometry

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The rate-governing step in the determination of the amino acid sequences of proteins is often the separation and purification of peptides rather than the actual determination of the sequences of the peptides. An approach has been made to decrease considerably the number of separation stages by applying the technique of mass spectrometry to the determination of the sequences of proteins. The use of mass spectrometry to the determination of the sequences of peptides was first suggested by Biemann *et al.* (1959) and was subsequently developed by several workers, with Morris (1970) showing that the sequences of peptides of unknown composition obtained from proteins and also mixtures of peptides could be determined by mass spectrometry.

Bovine α -lactalbumin is a milk protein whose sequence was fully determined by Brew *et al.* (1970). Tryptic and chymotryptic digests of the protein were partially separated on a Sephadex G-25 column, and the fractions taken were analysed by mass spectrometry. Although the separation stage does not yield a good separation, it does separate the peptides in the digest into groups of approximately the same molecular weight; and the weight of the sample to be analysed is the main factor governing the volatility of a sample, which itself determines whether a sample can be analysed by mass spectrometry. The volatility of each component of a mixture, however, will not be exactly the same, and this can be exploited by applying a temperature gradient to the sample inside the mass spectrometer. Samples of molecular weight up to about 1000 can be analysed completely, whereas those of larger size can have their sequences partially determined or be further digested to yield smaller peptides.

Although the peptides had been obtained from a protein source and had been converted into derivatives, no difficulties were experienced with impurities, for these tend to be more volatile than the peptides themselves and so can be removed at a low temperature. All the samples taken from the digests of α -lactalbumin were of completely unknown amino acid composition and therefore of unknown sequence, and the results were found to compare very favourably with the sequence published by Brew *et al.* (1970).

Three consecutive low-molecular-weight fractions from a Sephadex G-25 separation of a chymotryptic digest of α -lactalbumin yielded the sequence of 30 amino acid residues, i.e. a quarter of the whole molecule, illustrating the relative rapidity with which

sequence information may be obtained by using mass spectrometry.

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Patterns of Microbial Threonine Catabolism: a Survey

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A total of 75 micro-organisms capable of growth on L-threonine as the major source of carbon and nitrogen has been isolated from soils and other materials. A survey of catabolic routes has included growth, micro-respirometric and enzymic measurements.

The occurrence of L-threonine aldolase in extracts of threonine-grown isolates (Morris, 1969) was rare (six cases). L-Threonine dehydrogenase (Neuberger & Tait, 1960), the synthesis of which was induced (specific activities up to 2000 nmol/min per mg of protein), predominated in most cases (46). Sixteen isolates possessed enzymes acting on threonine that were either of low activity (less than 50 nmol/min per mg of protein) or undetectable when assayed under standard conditions. A fourth group of four isolates possessed only low threonine dehydrogenase activity but relatively high L-threonine dehydratase (deaminating) activity (Umbarger & Brown, 1957), which was constitutive. Another group (three isolates) possessed both threonine dehydrogenase and threonine dehydratase activities.

Classification of isolates was carried out by using numerical taxonomic procedures (Sokal & Sneath, 1963), and they were identified by standard diagnostic testing methods. To date, genus names have been assigned to the one-third of the isolates examined in detail. The largest group of strains was made up mostly of *Brevibacterium* spp., which possessed high threonine dehydrogenase activity after growth on L-threonine. Named strains, e.g. *Brevibacterium divaricatum* N.C.I.B. 9379 and *Brevibacterium linens* N.C.I.B. 8546, also grew well on threonine medium supplemented with 0.05% of yeast extract, and possessed high threonine dehydrogenase activity. Other

isolates in the same group included *Achromobacter* and *Flavobacterium* species.

Isolates with apparently low enzyme activities towards L-threonine, suspected to utilize novel routes of threonine catabolism or possess enzymes with atypical properties, included *Pseudomonas*, *Alcaligenes* and perhaps *Corynebacterium* species.

The further metabolism of the C₂ fragments of threonine catabolism catalysed by threonine aldolase or by threonine dehydrogenase plus 2-amino-3-oxobutyrate-CoA ligase (McGilvray & Morris, 1969; Blackmore & Turner, 1971) was also studied. Measurements of glyoxylate carboligase and serine dehydratase activities, diagnostic of routes for glycine catabolism via the glycerate pathway and the serine pathway respectively, permitted further subdivision of the isolates. One isolate, metabolizing threonine via 2-oxobutyrate and propionate, appeared to employ a constitutive 'biosynthetic' threonine dehydratase (Umbarger & Brown, 1957) to catalyse the initial step. Preliminary results of the survey show strong correlation between classifications according to classical taxonomic procedures on the one hand and enzymic analysis of catabolic pathways on the other.

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Utilization of Pairs of Substrates by Bacterium N.C.I.B. 8250 Growing in Batch Culture

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The induction and co-ordinacy of the enzymes involved in mandelate oxidation by bacterium N.C.I.B. 8250 have been the subject of previous investigations (Kennedy & Fewson, 1968a,b; Livingstone & Fewson, 1971). We have now used the patterns of growth on pairs of substrates as indications of other aspects of control such as repression and permeation.

Diauxic growth was observed when cells previously grown on benzoate were inoculated into benzoate+mandelate-salts medium. Benzoate was utilized first, and mandelate oxidation started only after the disappearance of benzoate. Inoculation of cells