None of the remaining 67 isolates utilized aminoacetone when washed suspensions of the bacteria grown on L-threonine were incubated under standard conditions (Higgins et al., 1968).

It was concluded that none of the 83 isolates metabolized L-threonine via aminoacetone. Additional work (S. C. Bell & J. M. Turner, unpublished work) suggests that all the selected isolates possessing high L-threonine 3-dehydrogenase activity catabolize 2-amino-3-oxobutyrate by cleavage to glycine and acetyl-CoA, as shown for a species of Arthrobacter (McGilvray & Morris, 1969). It seems unlikely that any commonly occurring bacterium degrades L-threonine by a route involving aminoacetone as an obligatory intermediate.

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The Effect of the Exogenous Folate Concentration on the Biosynthesis of Pteroylpolyglutamates in Lactobacillus casei and the Folate Polyglutamate Distribution in Escherichia coli

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It has been recognized for some time that most intracellular folate is attached to several glutamyl residues (Rabinowitz, 1962). An early study on yeast indicated that its folate was attached to a total of seven glutamyl residues (Pfiffner et al., 1946), although this organism has now been shown to contain some hexa- and octo-polyglutamates also (Bassett et al., 1976). Studies on mammalian folate polyglutamate distribution indicate that it is principally a mixture of penta-, hexa- and hepta-polyglutamates (Brown et al., 1974a; Shin et al., 1974; Leslie & Baugh, 1974), although the exact ratio of the three in the fully equilibrated endogenous state has only been determined in one instance (Brown et al., 1974a). The folate polyglutamate distribution has also been determined for Lactobacillus casei and Streptococcus faecalis by the same three groups (Brown et al., 1974b; Buehring et al., 1974; Baugh et al., 1974). Our own studies (Brown et al., 1974b) and those of Buehring and his co-workers (Buehring et al., 1974) have reported finding very-long-chain folate polyglutamates (greater than hexa) in L. casei, in sharp contrast with the findings of Baugh et al. (1974), who find that this organism contains principally pteroyl tetraglutamates with some pteroylpentaglutamates. Adding further to the controversy is that whereas Buehring et al. (1974) carried out their distribution studies on the intact folates by the method of Shin et al. (1972), our own studies and those of Baugh et al. (1974) were done after the folates present had been converted into the corresponding p-aminobenzoyl polyglutamates in a manner similar to that described originally by Houlihan & Scott (1972). The only significant difference between the three
studies was that owing to the low specific radioactivity of the radioactive pteroylglutamate (PteGlu) used by Baugh et al. (1974), the concentration of exogenous folate used by them in their medium was several thousand times that used in the other two studies. We have re-examined the folate polyglutamate distribution in L. casei (N.C.I.B. 10463), using different concentrations of exogenous \([^{3}H]\)PteGlu and have found that at higher concentration longer-chain folate polyglutamates are not formed.

The folate polyglutamate distribution of *Escherichia coli* was also determined for both wild-type (K12) and a p-aminobenzoate-requiring mutant (N.C.I.B. 8109).

The materials and methods used were essentially as previously described (Houlihan & Scott, 1972; Brown et al., 1974b). The following radiochemicals were supplied by The Radiochemical Centre, Amersham, U.K.: \([3,5,9\-(n)\-^{3}H]\)PteGlu at 5 Ci/mmol; PteGlu-\([^{14}C]\)Glu, PteGlu-\([^{14}C]\)Glu, PteGlu-\([^{14}C]\)Glu, PteGlu-\([^{14}C]\)Glu, all at 1–1.2 mCi/mmol.

Inocula of *L. casei* were prepared by growing a freeze-dried culture of the organism in 4 ml of nutrient broth to the exponential phase; 1 ml of this culture was used as inoculum for 200 ml of medium in 500 ml Erlenmeyer flasks. The bacteria were grown at 37°C with shaking for 36 h, by which time they were in the stationary phase. In all cases commercial folate-free media, supplied by BBL Laboratories, Cockeysville, MD, U.S.A. was used. Different concentrations of labelled and unlabelled PteGlu were added at the start of the incubation, giving final exogenous folate concentrations of 0.441, 7.056, 172.056, 337.056 and 1657.056 ng/ml.

*E. coli* inocula were prepared as follows. A freeze-dried culture of the mutant *E. coli* (N.C.I.B. 8109) was grown in 4 ml of nutrient broth overnight at 37°C; 1 ml of this was used to inoculate 150 ml of medium in an Erlenmeyer flask. The K12 strain of *E. coli* was sub-cultured from an agar plate and also grown overnight in 4 ml of nutrient broth, and 1 ml of this culture was used to inoculate 150 ml of medium in an Erlenmeyer flask and growth was for 24 h at 37°C (Brown et al., 1974b). The uptake of added radioactive compound was calculated by measuring the radioactivity in the starting medium and that remaining after the cells had been removed by centrifugation. Both organisms were harvested by centrifugation and washed twice in 0.9 % NaCl to remove any label attached to the outsides of the cells. In all cases the bacteria grew well and incorporated sufficient label for extraction of the pteroylpolyglutamates to be feasible.

Columns were calibrated by running either the radioactive folate polyglutamates described above or non-radioactive folate polyglutamates (Houlihan & Scott, 1972) prepared by the method of Krumdieck & Baugh (1969).

For *L. casei*, the profile of the folate polyglutamates at low exogenous concentrations (0.56 ng/ml) of label agrees with that already reported (Brown et al., 1974b; Buehring et al., 1974; Scott et al., 1975), in that major polyglutamates present were found to be the hexa-, hepta-, octo- and nona-glutamates. When the concentration of folate in the medium was increased the pattern obtained indicated a shift to shorter polyglutamyl chains.

The folate distribution patterns of *E. coli* were very similar for the mutant and wild type (K12), showing the following percentage distribution of polyglutamyl derivatives: mutant (N.C.I.B. 8109): 13% (mono); 18% (di); 30% (tri); 13% (tetra); 20% (penta); 5% (hexa); 1% (hepta); and wild-type (K12): 13% (mono); 14% (di); 36% (tri); 12% (tetra); 16% (penta); 8% (hexa); 14% (hepta).

The results indicate that the pteroylpolyglutamate pattern observed, in *L. casei* at least, is dependent on the concentration of exogenous label. Large amounts of exogenous folate in the case of *L. casei* caused shorter pteroylpolyglutamates to be formed than with smaller amounts of label, perhaps because of overloading the biosynthetic system. The possibility of being able to induce synthesis of either long- or short-chain folate polyglutamates by altering the growth conditions may prove useful in determining the function of these important cofactors.

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Purification and Properties of an Unusual Nitrilase from *Nocardia* N.C.I.B. 11216

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Little is known of the metabolism of the nitrile group by micro-organisms. Robinson & Hook (1964) reported the isolation of a nitrilase enzyme of rather restricted substrate specificity from a *Pseudomonas* sp. which would grow on the naturally occurring cyanopyridine, ricinine, and Mimura et al. (1969) have described a *Corynebacterium* sp. which can use aliphatic nitriles such as acetonitrile as sole carbon source. The nitrilase produced by this organism hydrolysates the nitrile to the corresponding amide.

In this communication the purification of a nitrilase from *Nocardia* N.C.I.B. 11216, which can use benzonitrile as sole carbon and nitrogen source, is described. Respiration studies on intact cells indicate that metabolism of benzonitrile proceeds through benzoic acid and catechol via the meta-cleavage route (Harper, 1974). Cell-free extracts were prepared by sonication of benzonitrile-grown cells suspended in 100 mM phosphate buffer, pH 7.5, containing 2 mM-EDTA and 2 mM-dithioerythritol, and centrifugation at 60000 g for 45 min. Such extracts show high activity in the conversion of benzonitrile into benzoic acid, the reaction being followed by assay of the NH₃ produced by using the phenol/hypochlorite method. Benzamide is not attacked and so does not appear to be a free intermediate in the reaction. The nitrilase was further purified by DEAE-cellulose column chromatography and passage through Sephadex G-200 in the presence and absence of benzonitrile. The resulting purified fraction was shown to be a single protein by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and isoelectric focusing on polyacrylamide gel.

On incubation with substrate the nitrilase shows a time-dependent activation process which is a function of enzyme concentration (Fig. 1). Neither benzoic acid nor ammonia had any effect on the length of the lag period. However, the activation process was highly dependent on both temperature and pH. A possible explanation of such behaviour is that the substrate, benzonitrile, causes association of inactive subunits to form the polymeric active enzyme. The molecular weight of the enzyme was therefore determined on Sephadex G-200 at 0°C in the absence of benzonitrile and also in the presence of 5 mM-benzonitrile after preincubation of the enzyme at 30°C for 4 min with 20 mM-benzonitrile. The elution behaviour of the enzyme under these conditions is shown in Fig. 2. In the absence of substrate the nitrilase has an elution volume corresponding to a mol.wt. of 47000, whereas in the presence of substrate the apparent mol.wt. increases to about 560000. The associated enzyme therefore seems to consist of about 12 identical inactive subunits. The molecular weight of the subunits is further confirmed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, which indicates a mol.wt. of 45000.