acetazolamide and were both decreased by ethosuximide (an effect previously observed with phenobarbitone). The thiol-group inhibitors iodoacetamide (100 μ M) and 5,5'-dithiobis-(2-nitrobenzoic acid) (100 μ M) did not alter the rate of xylose uptake over a 9 min incubation period. However, both inhibitors abolished the stimulatory effects of acetazolamide and ethosuximide.

The significance of these results will be discussed.

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Microbial Metabolism of Alkylbenzenesulphonates

By A. J. WILLETTTS and R. B. CAIN. (*Microbiology Group, Department of Botany, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 7RU, U.K.*)

Alkylbenzenesulphonates are a group of detergent compounds in common domestic and industrial usage. Several reports (Nelson, McKinney, McAteer & Konecky, 1961; Huddleston & Allred, 1963; Swisher, 1963) have concluded that this group of substituted aromatic compounds is biodegraded, but previous research has almost exclusively been limited to whole-cell studies with a mixed microflora degrading, in many instances, an isomeric mixture of alkylbenzenesulphonates. This communication reports the enzymology of (1-phenylundecyl)benzene-*p*-sulphonate degradation by a pure culture of a *Bacillus* sp. elected for growth on alkylbenzenesulphonates as the major source of carbon and sulphur.

Continuous sampling experiments, after inoculation of the pure strain of *Bacillus* species into alkylbenzenesulphonate medium, indicated that the sequence of biochemical events was: (1) removal of the sulphonate grouping, probably at the cell surface, leaving free sulphite and a *p*-hydroxyphenylalkanoic acid in the medium; (2) assimilation of sulphite and its subsequent intracellular oxidation to sulphate plus reduction to sulphide; (3) assimilation of the *p*-hydroxyphenylalkanoic acid, which was subsequently degraded intracellularly by classical β -oxidation of the alkyl side chain followed by 'ortho' cleavage of the aromatic nucleus (Ornston & Stanier, 1964).

The specific activity of the desulphonating enzyme, which was assayed by following sulphite production (Robinson, 1965), was increased 100-fold by growth on (1-phenylundecyl)benzene-*p*-sulphonate over that observed in succinate-grown cells. The enzyme was repressed but not inhibited

by sulphite in the medium. Substrate-specificity studies implicated isosteric activation of the enzyme. Sulphite-cytochrome *c* oxidoreductase activities were also elevated sixfold after growth on alkylbenzenesulphonates, but adenosine phosphosulphate-cytochrome *c* reductase was constitutive in this *Bacillus*.

Enzymes associated with β -oxidation were recorded in alkylbenzenesulphonate-grown cells. The acyl-CoA acyltransferase present was active with a range of detergent isomers and β -hydroxyacyl-CoA dehydrogenase was also detected. The activity of isocitrate lyase, an enzyme characteristically induced in metabolic sequences producing acetyl-CoA, was 90-fold higher in alkylbenzenesulphonate-grown than in succinate-grown cells.

The aromatic nucleus produced by biodegradation of alkylbenzenesulphonate homologues containing an even number of carbon atoms in the side chain was identified by g.l.c. and t.l.c. as *p*-hydroxyphenylacetate. The equivalent aromatic nucleus from homologues with an odd number of carbon atoms in the side chain was identified as *p*-hydroxybenzoate. All the enzymes to degrade the appropriate aromatic by a classical 'ortho' pathway to succinate and acetate were induced to high activities in *Bacillus* after growth on the appropriate alkylbenzenesulphonate isomer.

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Metabolism of Aromatic Compounds by Fungi: Conversion of β -Carboxymuconolactone into 3-Oxoadipate in *Aspergillus niger*

By D. R. THATCHER and R. B. CAIN. (*Microbiology Group, Department of Botany, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.*)

The conversion of protocatechuate into 3-oxoadipate was first shown to proceed via β -carboxymuconolactone [3-carboxy-4-hydroxyhex-2-enedioic acid (1 $\rightarrow$ 4)-lactone] in *Neurospora crassa* (Gross, Gafford & Tatum, 1956) and later incriminated as the lactone intermediate characteristic of fungal aromatic metabolism by Cain, Bilton & Darrah (1968). Prokaryotic organisms neither form β -carboxymuconolactone nor do they

utilize it, but dissimilate protocatechuate through the now well-characterized pathway involving the isomeric γ -carboxymuconolactone and its decarboxylation product 3-oxoadipate enol-lactone. Fungi show either no or very low activities with these lactones.

The conversion of β -carboxymuconolactone into 3-oxoadipate thus involves both decarboxylation and hydrolysis, but no intermediate has ever been implicated and this step remains biochemically ill-defined.

We have now purified the enzyme responsible for this conversion in *Aspergillus niger* some 250-fold, at which stage it was homogeneous on polyacrylamide-gel electrophoresis, all the enzyme activity being associated with the single protein band of molecular weight $54\,000 \pm 5000$. The pure enzyme produces stoichiometric amounts of 3-oxoadipate, CO_2 and protons, all at identical rates, from β -carboxymuconolactone with no detectable intermediate formation:

The enzyme displayed classical Michaelis-Menton kinetics with K_m $70\,\mu\text{M}$ at the pH optimum of 8.6; it was inactive with γ -carboxymuconolactone as substrate, but hydrolysed 3-oxoadipate enol-lactone and muconolactone at 9% and 6% respectively of the rate with β -carboxymuconolactone. The purified enzyme had a half-life of less than 24h unless stabilized with glycerol (20%), 2-mercaptoethanol (0.01%) and certain non-substrate analogues, from among which butyrolactone was used as a routine during purification.

These lactones act as competitive inhibitors. Cyanide (K_i 0.1mM), iodoacetate (K_i 0.14mM) and arsenite (K_i 0.8mM) are potent non-competitive inhibitors of the enzyme. A threefold decrease in K_m in the region of pH 8.3–8.7 (Benesch & Benesch, 1955) together with the requirement for 2-mercaptoethanol and the iodoacetate inhibition strongly suggest the presence of an active-site thiol group in this enzyme. Temperatures above 35°C rapidly inactivate the enzyme and Arrhenius plots show an unusual inflexion at 18.5°C . There is also some evidence for the presence of a tightly bound bivalent metal ion.

We conclude that the conversion of β -carboxymuconolactone into 3-oxoadipate is mediated by a single enzyme, 3-carboxy-4-carboxymethyl-4-hydroxycrotonolactone hydrolase (decarboxylating), with both delactonizing and decarboxylase activities.

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Tissue Distribution of Cholecalciferol (Vitamin D₃) in the Rat

By CARLENE A. HOLMAN, E. BARBARA MAWER and D. J. SMITH. (*Division of Metabolism, Department of Medicine, University of Manchester, York Place, Manchester M13 0JJ, U.K.*)

Although cholecalciferol is known to exert a biological effect long after administration of the vitamin has ceased, information on tissue storage sites is sparse.

Reports by Callow, Kodicek & Thompson (1966) and by Neville & DeLuca (1966) indicated that, in the rat, the liver contained much of the radioactivity during the first 2 days after administration of labelled cholecalciferol. We have extended this period of observation and now report changes in the distribution of radioactive cholecalciferol and its metabolites occurring during the first week after administration.

Mature rats that were not vitamin D-deficient were injected intracardially with 5i.u. (125ng) of [1,2- $^3\text{H}_2$]cholecalciferol and killed in pairs at intervals from 20min to 1 week after injection. Lipid extracts of tissues were made and chromatographed on silicic acid to separate cholecalciferol and its three principal more-polar metabolites (Mawer & Backhouse, 1969).

The results for distribution of the dose are expressed as the percentage of injected radioactivity that was present in each tissue when extrapolated to the whole body content.

Three groups of tissues were identified: (a) tissues with high early concentrations of radioactivity and rapid decline; liver and serum contained 70% and 11% respectively of the dose at 20min and 1.5% and 4% at 1 week; (b) tissues in which accumulation and decrease of radioactivity were slower; intestinal mucosa, kidney, bone and muscle contained at 1 week 0.05, 0.7, 1.6 and 3.8% respectively of the injected radioactivity; (c) tissues in which the radioactivity rose slowly and showed no decline; adipose tissue contained about 10% of the dose at 1 week. Some 80% of the radioactivity in liver, serum and mucosa, at 1 week, was present as more-polar metabolites, but in kidney, adipose tissue and muscle 70% of the recovered radioactivity was present as cholecalciferol.

These results suggest that, although the liver is