glycine concentration may develop across the cell membrane when $[Na^+]$ and $[K^+]$ in the two phases are appropriately manipulated in circumstances where the spontaneous movements of the ions across the cell membrane appear to provide the energy needed to concentrate the amino acid (Eddy, 1968a,b). Analogous observations have now been made in a modified system containing 2mM-sodium cyanide to inhibit respiration and 10mM-2-deoxyglucose to trap ATP. The accumulation of L-alanine, 2-aminoisobutyric acid, L-leucine, L-phenylalanine and L-methionine has been studied in the modified system at both large and small values of cellular and extracellular $[Na^+]$. The results, though less detailed than those previously obtained with glycine, encourage the view that similar mechanisms are involved in the transport of all these amino acids.

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The 'Active' Accumulation of Tetracycline by *Escherichia coli*

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The tetracycline antibiotics inhibit protein biosynthesis in cell-free preparations from both mammalian and bacterial cells (Franklin, 1963). The selective antimicrobial activity of these drugs *in vivo* may therefore depend in part at least on the curious ability of bacterial cells to accumulate them to high intracellular concentrations by an energy-dependent 'active' process (Izaki & Arima, 1963; Franklin & Godfrey, 1965; Franklin, 1967), which apparently does not occur in mammalian cells (Franklin, 1966).

The 'active' accumulation of a toxic substance not obviously structurally related to any known nutrient is a remarkable phenomenon and it has been found to have a number of unusual features. The initial rate of uptake of $[^3H]$ tetracycline by a tetracycline-sensitive strain of *Escherichia coli* is linearly dependent on the concentration of the drug in the medium, and no trend towards a saturation effect has been found at tetracycline concentrations up to approximately 1mM. Wilbrandt & Rosenberg (1961) pointed out that such linear kinetics do not preclude the possibility of a carrier-mediated transport if it is assumed that the affinity of the postulated carrier for the transported solute is very low.

When *E. coli* cells are incubated with low concentrations of $[^3H]$ tetracycline, e.g. 10 $\mu g./ml.$, the

accumulation of drug reaches a plateau in 7–10min. If the cells are then transferred to drug-free medium the label is rapidly lost from the cells. This exit reaction is markedly temperature-dependent, and is accelerated by the addition of 1mM-2,4-dinitrophenol. In contrast, when cells are preincubated with $[^3H]$ tetracycline at concentrations greater than 50 $\mu g./ml.$, uptake of the drug remains approximately linear for about 1hr. When these cells are transferred to drug-free medium, the rate of the exit reaction is found to be strikingly decreased compared with that in cells preincubated with lower concentrations of drug. Indeed, when the cells are preincubated with 200 $\mu g.$ of $[^3H]$ tetracycline/ml. the exit reaction is negligible, although loss of label from these cells can be readily provoked by dinitrophenol at 37°. It seems probable therefore that the intracellular accumulation of tetracycline above a certain concentration results in a progressive inhibition of the exit reaction. This in turn accounts for the continued uptake of the drug by *E. coli* when the extracellular drug concentration is greater than 50 $\mu g./l.$

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The Degradation of 2-Thiophenecarboxylic Acid by a *Flavobacterium* Species

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The thiophen ring is of restricted distribution in Nature, though a number of polythienyls have been isolated from plants in recent years (Gronowitz, 1963). However, little attention has been devoted to the microbiological dissimilation of thiophen (Knecht, 1961; Isenberg, 1961).

Attempts were made to isolate by enrichment culture micro-organisms able to degrade simple thiophens, but such attempts were unsuccessful. However, by using low (<0.5mM) concentrations of the enrichment substrate, a micro-organism was isolated that was able to grow in a simple mineral-salts medium with thiophen-2-carboxylate as sole source of organic carbon, and was provisionally identified as a *Flavobacterium* sp.

When the organism was grown in succinate medium, washed cells were able to oxidize thiophen-2-carboxylate at 0.1 $\mu mole/mg.$ dry wt. of cells/hr. in the presence of chloramphenicol at a concentra-

tion (100 $\mu\text{g./ml.}$) that prevented growth of the organism. Washed cells of the organism grown in a 2mm - thiophen - 2 - carboxylate - mineral - salts medium or in a succinate medium to which 2mm - thiophen-2-carboxylate had been added during growth were able to oxidize thiophen-2-carboxylate at rates up to fifteen times the corresponding rate of oxidation of the substrate by cells grown in the absence of thiophen-2-carboxylate, suggesting the operation of an inducible enzyme system. Whole cells of the organism grown in the presence of thiophen-2-carboxylate also showed corresponding increases in their ability to oxidize furan-2-carboxylate and pyrrole-2-carboxylate, and the three heterocyclic acids were also oxidized at increased rates by whole cells grown in a succinate medium in the presence of thiophen-3-carboxylate, which was not itself oxidized by induced or non-induced cells of the organism.

Washed cells of the *Flavobacterium* sp. grown in the presence of thiophen-2-carboxylate oxidized furan-2-carboxylate in the presence of 1mM-arsenite with the accumulation of 2-oxoglutarate, which did not accumulate during the arsenite-inhibited oxidation of thiophen-2-carboxylate, and thiophen-2-carboxylate (but not thiophen-3-carboxylate) was shown to prevent the accumulation of 2-oxoglutarate during the arsenite-inhibited oxidation of furan-2-carboxylate.

The failure of 2-oxoglutarate to accumulate during the arsenite-inhibited oxidation of thiophen-2-carboxylate was shown probably to result from the intracellular formation of a non-inhibitory compound (possibly As_4S_6) from the arsenite and sulphide, the latter previously having been shown to be a product of the degradation of thiophen-2-carboxylate by the *Flavobacterium* sp.

This work was supported by British Petroleum (Llandarcy) Ltd., whose help is gratefully acknowledged.

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The Competence-Inducing Factor of *Bacillus subtilis*

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A competence-inducing factor, which promoted the uptake of transforming DNA by certain strains of bacteria, was found in culture supernatants and aqueous extracts of competent cells of *Bacillus subtilis* strain 168 I⁻ (Charpak & Dedonder, 1965;

Akrigg, Atkinson, Ayad & Barker, 1967). The competence-inducing factor appeared to be polypeptide in nature, since its activity was abolished by treatment with trypsin.

Aqueous extracts of competent cells of *B. subtilis* were fractionated by DEAE-cellulose chromatography (Akrigg, Ayad & Barker, 1967). Five peaks showing extinction at 280m μ were obtained. Of these, only peak 2 had the property of provoking competence in non-competent cells.

Young & Spizizen (1963) found an autolytic enzyme associated with the cell walls of a highly transformable strain of *B. subtilis*. Cell walls from poorly transformable strains showed a lower activity of the enzyme.

In our recent work, cell walls of *B. subtilis* strain 168 I⁻ were prepared by the ultrasonic treatment of cells grown for 4hr. in medium I (Akrigg, Ayad & Barker, 1967). Cell walls were separated from the remaining whole cells by centrifugation at 1000g for 10min. The native autolytic enzyme associated with the purified cell walls was inactivated by heating the cell-wall suspension at 80° for 30min. Heat-inactivated cell walls could be lysed by incubation at 37° at pH 8.3, with a sample of a crude autolysate of cell walls containing the active autolytic enzyme [a modification of the method of Young & Spizizen (1963)].

Aqueous extracts of competent cells of *B. subtilis* strain 168 I⁻ were fractionated on DEAE-cellulose, with either linear or convex gradients of NaCl for elution. Samples of each of the five ultraviolet-absorbing peaks obtained were incubated with heat-inactivated cell walls as described above.

It was observed that when heat-inactivated cell walls were incubated with peak 2, lysis occurred. No lytic activity was exhibited by any of the other peaks.

The results suggest that, the polypeptide material present in peak 2, which can induce competence in non-competent cells, may also be the lytic enzyme described above.

The authors thank Professor G. R. Barker for helpful discussion and for providing the facilities for this work. A. A. is indebted to the Science Research Council for the award of a studentship.

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