labelled with [^{131}I]iodine by the method of Landon, Livanou & Greenwood (1967) at specific radioactivities of $500\ \mu\text{Ci}/\mu\text{g}$. The injected solution also contained ^{125}I -labelled plasma albumin or [^{125}I]iodide. The animals were then killed at various intervals and the radioactivity due to both isotopes in a portion of each tissue was measured in an automatic spectrometer.

^{131}I -labelled adrenocorticotrophic hormone disappeared rapidly from the plasma and was concentrated mainly in the kidney. By contrast neither [^{125}I]iodide nor ^{125}I -labelled albumin was found to accumulate at this site. There was no evidence for preferential adrenal uptake of either radioactive isotope. Extraction of tissues for adrenocorticotrophic hormone by the method of Richards & Sayers (1951) showed that a significant proportion of the renal ^{131}I radioactivity had the physicochemical characteristics of the hormone.

In similar experiments the intra-adrenal distribution of radioactivity was determined after serial sectioning of the frozen gland (Grunbaum, Geary & Glick, 1956). ^{131}I -labelled adrenocorticotrophic hormone was concentrated in the glomerulofascicular border and in the reticular zone. On the other hand ^{125}I -labelled albumin was found mainly in the reticular zone, and [^{125}I]iodide was uniformly distributed throughout the gland.

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Grunbaum, B. W., Geary, J. R. & Glick, D. (1956). *J. Histochem. Cytochem.* **4**, 555.

Landon, J., Livanou, T. & Greenwood, F. C. (1967). *Biochem. J.* **105**, 1075.

Richards, J. B. & Sayers, G. (1951). *Proc. Soc. exp. Biol. Med.* **77**, 87.

Sonenberg, M., Keston, K. S. & Money, W. L. (1951). *Endocrinology*, **48**, 148.

Membrane-Bound Penicillinase of *Bacillus licheniformis* 749/C

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In *Bacillus licheniformis* 749/C there is apparently only one structural gene for penicillinase although the enzyme is found in the growth medium and in the membrane (Dubnau & Pollock, 1965). The extracellular form is soluble and the amino acid sequence is known (Ambler & Meadway, 1969). It has proved difficult to solubilize the cell-bound form by using agents that disrupt only non-covalent bonds (Lampen, 1967), although trypsin releases

the enzyme in a form that is similar to the exo form except for the absence of the terminal lysine residue (Ambler & Meadway, 1969). As the two forms are so closely related, comparative studies were undertaken to reveal properties of importance in determining the cell-bound state.

The intracellular enzyme is distributed approximately equally between the two subcellular membranous fractions (a) the plasma membrane and (b) a vesicle fraction released from cells on preparing protoplasts. They are also differentiated by their role in the secretion of penicillinase (Sargent, Ghosh & Lampen, 1968, 1969).

Both intracellular forms of penicillinase were solubilized in 0.1% sodium deoxycholate and 0.05M-pyrophosphate buffer, pH 9.0. Previous studies suggested the enzyme would remain particulate after removal of deoxycholate, and therefore the molecular weight in the presence and absence of deoxycholate was determined by gel filtration on Bio-Gel A-5M, with columns calibrated with protein standards, which were run with the sample or in a consecutive experiment.

Both cellular forms of penicillinase are eluted in the presence of deoxycholate with a molecular weight of 45000. In the absence of deoxycholate plasma-membrane penicillinase is eluted at a molecular weight of 600000 whereas vesicle-fraction penicillinase is eluted at a molecular weight of 24000 (i.e. identical with that of the exo form), and deoxycholate is separated from the proteins. Both changes in molecular weight are readily reversible. In contrast the elution volume of exoenzyme and four other proteins is unchanged in the presence or absence of deoxycholate.

Ammonium sulphate, pH 8.0 (25%, w/v), added to deoxycholate solubilizates of plasma membrane converts the plasma-membrane form into the exo form by a time- and temperature-dependent reaction. Vesicle-fraction penicillinase is converted into the exo form at pH 9.0 without salt in the absence of deoxycholate after partial purification. As these conditions are unlikely to involve changes in covalent bonding, the two cell-bound forms of penicillinase probably represent conformational variants of the exo form. The vesicle-fraction penicillinase we suggest is a hydrophobic but monomeric variant of the exo form, which in the presence of deoxycholate binds up to 50 molecules of deoxycholate/molecule to reach a molecular weight of 45000. The plasma-membrane form we consider to be an aggregate of 25 molecules that depolymerizes to monomers in the presence of deoxycholate but that then bind deoxycholate on their hydrophobic faces in the manner suggested for the vesicle-fraction enzyme.

The results certainly indicate marked organizational differences between the soluble exo form and

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the cell-bound forms that may result from conformational differences that enable the enzyme to be cell-bound.

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- Ambler, R. P. & Meadway, R. J. (1969). *Nature, Lond.*, **222**, 24.
 Dubnau, D. A. & Pollock, M. R. (1965). *J. gen. Microbiol.* **41**, 7.
 Lampen, J. O. (1967). *J. gen. Microbiol.* **48**, 249.
 Sargent, M. G., Ghosh, B. K. & Lampen, J. O. (1968). *J. Bact.* **96**, 1231.
 Sargent, M. G., Ghosh, B. K. & Lampen, J. O. (1969). *J. Bact.* **97**, 820.

The Regulation by Methionine of Three Inducible Enzymes in *Coprinus lagopus*

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Coprinus lagopus is a basidiomycete fungus with many genetic advantages over other fungi in the elucidation of systems regulating the synthesis of specific enzymes (Lewis, 1961; Casselton & Lewis, 1967). Wild-type strains of *Coprinus* may be grown aerobically in a simple defined medium.

The synthesis of three enzymes has been found to be regulated by methionine: *S*-adenosylmethionine synthetase (ATP-methionine *S*-adenosyltransferase, EC 2.5.1.6) and two methionine decarboxylases (L-methionine carboxy-lyase, EC 4.1.1.-), with pH optima at pH 7.8 and pH 4.6. These enzymes were assayed by using ¹⁴C-labelled methionine as substrate and trapping any labelled cationic product (*S*-adenosylmethionine or 3-methylthiopropylamine) on CM-cellulose paper discs.

For all assays samples were spotted on to CM-cellulose paper discs impregnated with 50 mM-L-methionine. The large molar excess of unlabelled substrate effectively stops the radioactive reaction while the discs are drying. The discs are then washed with distilled water to remove unchanged substrate, dried and their radioactivities measured in a liquid-scintillation counter as described by Newsholme, Robinson & Taylor (1967).

The methionine decarboxylases were assayed with L-[Me-¹⁴C]methionine as substrate. Because the decarboxylases also produce a labelled cation from L-[Me-¹⁴C]methionine, *S*-adenosylmethionine synthetase cannot be measured easily with this as a substrate. Instead, L-[carboxy-¹⁴C]methionine was used as substrate, any decarboxylation resulted in unlabelled product, the label thus being incorporated into *S*-adenosylmethionine only.

It was found that the two methionine decarboxy-

lases were repressed by adding methionine to the growth medium, whereas the *S*-adenosylmethionine synthetase was induced. Ethionine, a toxic analogue of methionine, could induce *S*-adenosylmethionine synthetase to only 60% of the methionine-induced activity, but could repress the decarboxylases much more effectively. Ethionine is a substrate for the decarboxylases but has a *K_m* 100-fold greater than that of methionine for *S*-adenosylmethionine synthetase. All the enzymes have been purified.

A possible mechanism is that the methionine decarboxylases are repressed by their product, and that charged methionine tRNA acts as the inducer for *S*-adenosylmethionine synthetase.

- Casselton, L. A. & Lewis, D. (1967). *Genet. Res.* **9**, 63.
 Lewis, D. (1961). *Genet. Res.* **2**, 141.
 Newsholme, E. A., Robinson, J. & Taylor, K. (1967). *Biochim. biophys. Acta*, **132**, 338.

Methylation of Homocysteine in *Coprinus lagopus*

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The basidiomycete *Coprinus lagopus* may be grown aerobically in simple defined media. Cell-free extracts form L-methionine from homocysteine when L-serine is used as ultimate source of the methyl group. The individual reactions involved have been studied in the light of the pathways established in, for example, *Escherichia coli* (Woods, Foster & Guest, 1964) and *Saccharomyces cerevisiae* (Botsford & Parks, 1967).

With extracts of *Coprinus* either tetrahydropteroyltriglutamate (H₄PteGlu₃) or a boiled extract of *E. coli*, containing natural polyglutamyl folates (Guest & Woods, 1962), can serve as the folate coenzyme carrying the C₁ unit. Tetrahydropteroylmonoglutamate (H₄PteGlu) is inactive.

An isotopic method has been developed for the determination of serine transhydroxymethylase (serine-tetrahydrofolate 5,10-hydroxymethyltransferase, EC 2.1.2.1). *Coprinus* extracts transfer C₁ fragments from L-serine to tetrahydrofolate at a rate sufficient to satisfy the overall production of methionine measured under similar conditions. Both H₄PteGlu and H₄PteGlu₃ were effective C₁ acceptors. *Coprinus* extracts contain N⁵N¹⁰-methylene tetrahydrofolate reductase activity (N⁵-methyltetrahydrofolate-FAD oxidoreductase, EC 1.1.1.68), forming N⁵-methyltetrahydrofolates. Small quantities of these extracts promote methio-