RELEASE OF ENZYMES FROM BACTERIA

D. F. Broad and J. T. Smith, Microbiology Section, Department of Pharmaceutics, School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, U.K.

β. lactamases are enzymes which commonly cause clinical strains of bacteria to resist the penicillin and cephalosporin antibiotics. Certain β. lactamases can be released from Gram-negative bacteria by a variety of techniques which disrupt the outer cell wall but do not cause cell lysis nor release cytoplasmic enzymes. On the other hand, some β. lactamases remain cell bound during these treatments. One such technique, osmotic shock, has been used to study the release of a variety of enzymes from E. coli. Enzymes released by this procedure have been assigned to a discreet cellular location termed the periplasm (Heppel, 1971) Those enzymes which are retained during osmotic shock have been assumed to have a different cellular location and they have been termed cell bound (Heppel, 1971). However, there is no evidence that enzymes exist in these two locations during normal conditions.

Neu (1968) studied the release of β. lactamases by osmotic shock treatment and concluded that R-factor mediated β. lactamases were periplasmic whereas chromosomally mediated ones were cell bound. However, results presented here (table) and by Wyatt and Smith (1974) indicate that it is the molecular weight of the enzyme, and not the genetic location of its structural gene, which determines whether or not a β. lactamase can be released by osmotic shock treatment; β. lactamases of molecular weight greater than 30,000 not being released.

β. lactamase	Genetic location*	Molecular weight	Percent. Release
R7268	R	20,500	96.2
RP1	R	19,500	94.8
RGN238	R	24,000	83.8
R22Ka	R	31,800	0
K. aerogenes 418	C	20,080	74.4
E. coli D3	C	31,800	0

* R represents R. factor and C represents Chromosomal β. lactamases

When the release of the β. lactamases mediated by R. factors R46 and R55 was studied it was found that despite having molecular weights of 44,600 and 41,200 they were released to some extent by osmotic shock (11.2% and 52.0%, respectively). This apparantly anomolous behaviour can be explained by the finding that these two enzymes are dimeric (Dale and Smith, 1976). Thus either these enzymes in the dimeric state are sufficiently asymmetric, or their individual sub-units are sufficiently small, to pass through the cell wall during osmotic shock.

The cell wall therefore appears to act simply as a molecular sieve during osmotic shock treatment and thus little or no conclusions regarding cellular location should be drawn from osmotic shock data.

Dale J.W. and Smith J.T. (1976) Biochem. Biophys. Res. Comm. 68, 100
Heppel L.A (1971) "Structure and Function of Biological Membranes " Ed. L. I. Rothfield p.223
Neu H.C. (1968) Biochem. Biophys. Res. Comm. 32, 258
Wyatt J.M. and Smith J.T. (1974) J.Bacteriol. 117,931