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Neu H C & Heppel L A. The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. *J. Biol. Chem.* 240:3685-92, 1965.
[National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, US Public Health Service, Bethesda, MD]

This paper describes the methods to release enzymes localized to the periplasmic space of *Escherichia coli*. This technique has been widely used to demonstrate the localization of enzymes and transport proteins to the periplasmic space of bacteria. It has been useful in isolating transport proteins, beta-lactamases, and aminoglycoside-inactivating enzymes. [The *SCI*® indicates that this paper has been cited in over 760 publications.]

Osmotic Shock of Bacteria

Harold C. Neu
Departments of Medicine and Pharmacology
College of Physicians & Surgeons
Columbia University
New York, NY 10032

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This work was conducted while I was a research associate in the National Institute of Arthritis and Metabolic Diseases (NIAMD). I had completed part of a residency in internal medicine at Presbyterian Hospital in New York City, and I had applied to come to the National Institutes of Health (NIH) to work as a research associate in NIAMD. I had interviewed with Leon A. Heppel and felt that he would be the perfect mentor for me since I wished to learn about nucleic acid biochemistry. Leon was everything a young person needed, since he was even more compulsive than I and willing to spend a number of hours each day initially tutoring me. After I had demonstrated I could calibrate Lang-Levy pipets, elute nucleotides, and use a balance, I began to develop a method to chemically sequence tRNA. The technique to achieve removal of one nucleotide at a time required a purified alkaline phosphatase and an exonuclease. I became interested in how these enzymes could be obtained most easily from bacteria. In the course of developing procedures to isolate the enzymes, we discovered that a recently described nuclease was released when bacteria were subjected to marked changes in osmotic environment. This suggested to us that the enzymes might be on the surface of the bacteria. Heppel and I published our findings on the RNase and a paper on the use of the periodate reaction to sequence long nucleotides.[1]

Few today understand how tedious was the work to remove one nucleotide per day and establish which one had been removed by paper elution chromatography. Quite by accident I discovered that there was another enzyme in the periplasmic space, namely, a 5'-nucleotidase that we subsequently showed acted as an ATPase and also hydrolyzed uridine diphosphate glucose.[2] The discovery of this enzyme was met with skepticism by the entire laboratory section, and I was assured by everyone that my observations were an artifact since I must have used the incorrect buffers. Finding that the 5'-nucleotidase and a 2',3'-cyclophosphodiesterase were released by what we now called osmotic shock clearly indicated that a number of enzymes must be in the periplasmic space. This provoked the work of this paper.

The osmotic-shock technique immediately saw use as a method to obtain and study proteins involved in the transport of amino acids into cells.[3,4] I returned to New York to become chief medical resident at Presbyterian Hospital, even though I would have preferred to remain in Bethesda working on the projects I had going. But it was another era. There may have been demonstrations and hippies, but in American medicine if one had promised two years before to do something, one had better do it if one wanted to survive in academic medicine. After finishing my chief residency, I became a Career Investigator of the New York City Health Research Council and, with an NIH grant, purified the 5'-nucleotidase.[2] Becoming an infectious disease person, I looked to see if the plasmid-mediated beta-lactamase was released by osmotic shock, and we subsequently used the technique to purify the beta-lactamase.[5] Other investigators subsequently used osmotic shock to obtain aminoglycoside-inactivating enzymes.[6] Over the years the technique has been employed to study enzymes of many bacterial species.[7]

This work and its wide acceptance was a major point in my career. It made it possible for me to purify a number of enzymes very rapidly. This work and the subsequent use of it by others and my own group to study antibiotic-inactivating enzymes as well as my subsequent work on antibiotics were factors in my receiving the Hoechst-Roussel Award of the American Society of Microbiology in 1983 and the Squibb Lectureship of Rutgers University.

I have remained at Columbia University. Dr. Heppel remains an active investigator and teacher at Cornell University and recently had a *Festschrift* for his 75 years. I still, and forever will, regard him as my scientific father.

I think this paper is frequently cited because it provided the method to use osmotic shock to obtain proteins in the periplasmic space of bacteria.[8] It is the optimal way to obtain beta-lactamases, aminoglycoside-inactivating enzymes, and transport proteins with only 60 to 100 proteins compared to over 2,000 when bacteria are disrupted, and it has led to research in a number of different areas concerned with how molecules enter and leave bacteria.

1. **Neu H C & Heppel L A.** Nucleotide sequence analysis of polyribonucleotides by means of periodate oxidation followed by cleavage with an amine. *J. Biol. Chem.* 239:2927-34, 1964. (Cited 140 times.)
2. **Neu H C.** The 5'-nucleotidase of *Escherichia coli*. I. Purification and properties. *J. Biol. Chem.* 242:3896-904, 1967. (Cited 105 times.)
3. **Heppel L A.** The concept of periplasmic enzymes. (Rothfield L I, ed.) *Structure and function of biological membranes*. New York: Academic Press, 1971. p. 233-47. (Cited 180 times.)
4. **Oxender D L.** Membrane transport proteins. (Manson L A, ed.) *Biomembranes*. New York: Plenum Press, 1974. Vol. 5. p. 25-9. (Cited 20 times.)
5. **Neu H C.** The surface localization of penicillinases in *Escherichia coli* and *Salmonella typhimurium*. *Biochem. Biophys. Res. Commun.* 32:258-63, 1968. (Cited 50 times.)
6. **Umezawa H, Takasawa S, Okanishi M & Utahara R.** Adenylyl-streptomycin, a product of streptomycin inactivated by *E. coli* carrying R-factor. *J. Antibiotics* 21:81-2, 1968. (Cited 85 times.)
7. **Odelson D A, Patterson M A & Hespell R B.** Periplasmic enzymes in *Bdellovibrio bacteriovorus* and *Bdellovibrio stolpii*. *J. Bacteriology* 151:756-63, 1982. (Cited 5 times.)
8. **Oliver D B.** Periplasm and protein secretion. (Neidhardt F C, Ingraham J L, Low K B, Magasanik B, Schaechter M & Umbarger H E, eds.) *Escherichia coli and Salmonella typhimurium. Volume 1. Cellular and molecular biology*. Washington, DC: American Society for Microbiology, 1987. p. 56-69.

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