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Schrauzer G N, White D A & Schneider C J. Cancer mortality correlation studies—III: statistical associations with dietary selenium intakes. *Bioinorg. Chem.* 7:23-34, 1977.

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This paper reports inverse correlations between the age-corrected mortalities from cancer at major body sites and selenium intake estimated from food consumption data and blood selenium levels with data for the US and different countries. The results support the hypothesis that selenium has cancer-protecting effects in humans. [The *SCI*® indicates that this paper has been cited in over 155 publications.]

Selenium and Cancer

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In 1967 I was interested in the mechanisms of oxidation-reduction reactions involving thiols as electron donors and organic dyes such as methylene blue as acceptors, as reactions of this type are catalyzed by traces of a variety of metals as well as by vitamin B_{12}.[1] In searching the literature for related reactions of biological relevance, I came across a historical "cancer test" that consisted of the measurement of the methylene blue reduction time by human plasma. Originally developed at Massachusetts General Hospital,[2] it was later widely used at New York Medical College.[3] The test was believed to measure free plasma sulfhydryl groups and was popular because it seemed to allow cancer diagnosis even in patients with microscopically small tumors. It was eventually abandoned, however, because it also produced false positive results.

Because of the susceptibility of methylene blue reduction reactions to accelerating effects by various elements, we suspected that the test really indicated the absence of a biogenic catalyst in the plasma of cancer patients. The missing catalyst turned out to be selenium, which in turn suggested that selenium was a physiological cancer-protecting agent.[4] As is true for many discoveries, they are often made simultaneously in several laboratories. In the present case, R.J. Shamberger and D.V. Frost reached similar conclusions after finding, contrary to expectation, lower cancer mortalities in high-selenium regions of the US than in areas deficient in the element.[5]

Life-term experiments with tumorvirus-infected C3H mice to prove the selenium-cancer protection hypothesis were begun shortly thereafter. These showed that mammary tumorigenesis was prevented by subtoxic amounts of selenium in the drinking water.[6] The result seemed relevant to humans in that excess breast cancer mortalities were observed in low selenium areas of the US.

Thanks to the generosity of Dr. Leonell C. Strong, additional life-term studies with C3H mice could be completed, confirming the previous findings. David A. White, a brilliant graduate student (who since has become an MD-PhD specializing in oncology), and his wife, Carol J. Schneider, an excellent secretary and laboratory technician, helped with much of the work. These outstanding coworkers also collected numerous blood and food samples from different states and countries for selenium analysis. As significant inverse associations were obtained between selenium intake variables and mortalities from cancers at major organ sites, it was postulated that cancer mortalities in the US and other Western industrialized countries could probably be lowered by increasing the dietary selenium intakes to 250-300 micrograms/day, approximately twice the current average American intakes.

The paper triggered widespread interest in selenium as a potential cancer-protecting agent. It also probably made some skeptics cringe, but the key conclusions still stand. Human selenium supplementation experiments to test the usefulness of selenium for human cancer prevention are presently in progress in the US and in China. Some promising results are already available: In a pilot study conducted with 20,847 subjects from a commune in Qidong, a county north of Shanghai, supplemental selenium reduced the incidence rate of viral hepatitis,[7] which constitutes a major liver cancer risk factor in this area. It was also found that the lymphocytes of subjects receiving selenium were more resistant against aflatoxin B, another liver cancer risk factor in the Qidong area.[8] In the US, under the direction of L.C. Clark, Arizona Cancer Center, Tucson, a controlled selenium supplementation study involving about 1,100 high-risk subjects is in progress to assess the value of selenium for skin cancer prevention. This study will be completed within the next one or two years and could well provide compelling evidence for the value of selenium in cancer prevention.

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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

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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.

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