

This Week's Citation Classic

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Lardy H A, Johnson D & McMurray W C. Antibiotics as tools for metabolic studies. I. A survey of toxic antibiotics in respiratory, phosphorylative and glycolytic systems. *Arch. Biochem. Biophys.* 78:587-97, 1958.
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Antibiotics toxic to animals were found to have specific inhibitory effects on mitochondrial metabolism—some as inhibitors of respiratory enzymes, others as inhibitors of phosphorylation (oligomycin), some as uncouplers or inhibitors of respiration specific for certain substrates. Subsequent work in many laboratories confirmed the prediction that 'toxic antibiotics might prove to be generally useful tools for investigating metabolic systems.' [The SC[®] indicates that this paper has been cited over 560 times since 1961.]

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"In the 1950s the field of oxidative phosphorylation was so nebulous and confused that all possible concepts, facilities, and tools were needed to advance knowledge of the subject. When Hotchkiss found that gramicidin blocked phosphate uptake by *Staphylococci*, we had postulated that this antibiotic uncoupled phosphorylation from oxidation—a concept we had first proposed for the metabolic effect of 2,4,-dinitrophenol (DNP).^{1,2} From then on, we collected antibiotics from many sources hoping that some would be useful inhibitors of cellular respiration and phosphorylation. The first of these found to be effective was usnic acid³ which, in micromolar concentrations, uncoupled phosphorylation in washed liver particles. We also discovered that antimycin A blocked the respiratory system of bakers yeast and enhanced aerobic fermentation to the anaerobic rate. This information was passed on to Ahmad and Strong in the next laboratory, who were working intensively on antimycin.

"By 1958 we had tested more than 60 antibiotics and had found about one of 10 to have interesting effects on mitochondrial metabolism and function. When we later confined our screening to antifungals that were non-toxic to anaerobic bacterial growth, one of every three or four new antibiotics was a 'keeper.'

"The 1958 paper reported the inhibition of mitochondrial respiration by oligomycin and its reversal by DNP. It inhibited ATP hydrolysis induced by DNP, Ca²⁺, deoxycholate, and triiodothyroacetic acid. We concluded that oligomycin acts on an enzyme involved in phosphate fixation or in phosphate transfer rather than on enzymes in electron transport. Regardless of which theory of oxidative phosphorylation an investigator espoused, he was soon finding oligomycin useful in his work. We kept no record of the number of requests we received for this antibiotic but I know we disposed of a gross of small vials before we began folding samples into glazed paper and sending them by letter mail.

"Valacidin (identical with Pfizer's streptonigrin) was found to reverse respiratory inhibition by oligomycin or antimycin 'indicating that it acts as an electron carrier from DPNH to cytochromes.'

"The paper also reported for the first time the effects of nigericin and dianemycin on mitochondrial respiration, phosphorylation, and ATP hydrolysis.

"Dianemycin was named for Diane Johnson, the second author of the paper. She is a 'classic' in her own right. After 10 years as my technician Johnson decided to take a doctorate in history of science. Although her undergraduate academic record was brilliant, as a Ph.D. candidate she suffered the disgrace of her only B grade—in a one-credit seminar. (The professor who awarded it has since left this University and I have wondered whether he was encouraged to do so by the assistant dean of his college—Diane Johnson.) She is also chairman of the Drug Quality Council for the State of Wisconsin—their task, to determine whether generic drug products are equivalent to brand name products. *Believe it or not*, she has just been appointed assistant director of athletics at the University of Wisconsin!

"Bill McMurray was a postdoctorate fellow from the University of Western Ontario, where he is now professor of biochemistry and where he later discovered the effects of valinomycin on mitochondria.

"I have always considered this one of the trivial papers from our laboratory but am pleased that others have found it useful enough to cite it."

1. Lardy H A & Elvehjem C A. Biological oxidations and reductions. *Ann. Rev. Biochem.* 14:1-30, 1945.
2. Lardy H A & Phillips P H. The effect of thyroxine and dinitrophenol on sperm metabolism. *J. Biol. Chem.* 149:177-82, 1943.
3. Johnson R B, Feldott G & Lardy H A. The mode of action of the antibiotic, usnic acid. *Arch. Biochem.* 28:317-23, 1950.