Effects of 2,4-Dinitrophenol upon Oxygen Consumption and Ciliary Activity in the Ctenidia of *Mytilus*.* (19778)

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Nitrated phenols such as 2,4-dinitrophenol (DNP) are known to have the ability to increase the consumption of oxygen and the production of heat by cells and tissues(1). Although the mechanism of action of DNP is obscure, it has been suggested that it "uncouples" the energy-yielding steps of metabolism from those which make use of the released energy(2). Direct observation of energy utilization by cells treated with DNP should contribute to knowledge of its mechanism of action. This report describes measurements of oxygen consumption and ciliary action in molluscan ciliated epithelium treated with DNP. The inhibition of phosphate uptake in this tissue by other chemical agents has been reported previously(3).

Materials and methods. Excised ctenidia (gills) of *Mytilus edulis* L., the edible mussel, were transferred to petri dishes of filtered sea water cooled to 20° or 21°C. The ctenidia were teased apart if necessary and cut into squares or rectangles about 5 mm long.

The DNP was first titrated with NaOH to convert it to its sodium salt. The stock solution of DNP, made in filtered sea water buffered at pH 8.0 with 0.03 M glycine, was diluted further with sea water for use.

Oxygen consumption was measured manometrically at 20°C in 15 ml Warburg flasks. Each flask contained 2 ml of buffered sea water and 3 or 4 ctenidial fragments. Ciliary activity was measured by observing the rate at which pieces of ctenidia moved across the bottom of a petri dish containing sea water or DNP solution(4). Each piece was timed first in sea water and then after immersion in DNP solution. Controls were run in sea water alone. The time required for the tissue fragment to move 0.5 cm was measured 5 times in 5 minutes, beginning 15 minutes after immersion; the observations were then converted to velocities and averaged.

Results and discussion. The results of the experiments are summarized in Table I. In general the data indicate that DNP augments oxygen uptake in excised *Mytilus* gill with a maximal effect at a concentration between 2.9×10^{-4} and 1.2×10^{-3} M. (Graphic interpolation gave 5×10^{-4} M as the most effective concentration of DNP). At the highest concentrations employed there appeared to be a return to normal (or perhaps subnormal) oxygen consumption. Similar maxima have been observed in a variety of animal tissues and microorganisms(1).

DNP had an inhibitory effect on ciliary beat. Inhibition was estimated to be 50% effective when the concentration of DNP was between 2.9×10^{-4} M and 5.8×10^{-4} M (graphic estimation giving 4×10^{-4} M as the concentration for 50% inhibition) and was complete in the presence of 7.7×10^{-3} M DNP. Thus the concentration at which the DNP caused the greatest change in ciliary activity coincides closely with the concentration at which it caused maximal augmentation of respiration.

The augmentation of energy release has been visualized by Peiss and Field(1) as the inactivation of a "brake" type of rate-controlling mechanism. Spiegelman and Kamen (5) have shown that in yeast, DNP causes a decrease in the rate of assimilation of phosphorus, probably into energy-rich phosphate bonds. Tyler(6) has suggested that there may be more than one site of action for DNP.

The results reported here suggest that oxidative breakdown of intracellular foods is accelerated by DNP at the same concentration that interrupts ciliary activity. Although our data do not provide additional support for the multiple action of DNP, they do suggest

*Aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Delaware (NR-160-015).

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TABLE I. Effects of DNP on Oxygen Consumption and Rate of Ciliary Activity of *Mytilus* Gill.

Final conc. DNP, mols • l^{-1}	Oxygen consumption, normal = 1		Ciliary activity, normal = 1	
	n	Mean and std error	n	Mean and std error
1.1 × 10^{-6}	4	.95 ± .03		
2.3 × 10^{-6}	4	.95 ± .01		
4.5 × 10^{-6}	4	.94 ± .02		
9 × 10^{-6}	4	.89 ± .05		
1.8 × 10^{-5}	4	1.08 ± .03	3	.91 ± .02
3.6 × 10^{-5}	4	.97 ± .05	3	.91 ± .06
7.2 × 10^{-5}	5	1.39 ± .02	3	.74 ± .21
1.4 × 10^{-4}	11	1.48 ± .05	3	.68 ± .15
2.9 × 10^{-4}	19	1.75 ± .07	3	.74 ± .02
5.8 × 10^{-4}	23	1.88 ± .02	3	.41 ± .07
1.2 × 10^{-3}	10	1.49 ± .05	3	.16 ± .04
2.3 × 10^{-3}	6	1.35 ± .06	3	.06 ± .03
4.6 × 10^{-3}	6	1.11 ± .02	3	.14 ± .05
7.7 × 10^{-3}			3	No activity

that the augmentation of respiration and the uncoupling of endergonic processes may be closely linked. Further studies of metabolism in ciliated cells may clarify the site of action of DNP.

Summary. 1. The effects of 2,4-dinitrophenol (DNP) upon oxygen consumption and ciliary activity in the excised ctenidia of *Mytilus edulis* were investigated. 2. The maximal stimulation of oxygen uptake was estimated to occur when the DNP was 5 x 10^{-4} M. 3. Ciliary beat was increasingly inhibited as the concentration of DNP was raised. 50% inhibition was estimated at 4 x 10^{-4} M DNP, and inhibition was complete in the presence of 7.7 x 10^{-3} M DNP. 4. The possible role of DNP in uncoupling exergonic from endergonic processes receives support from the close coincidence of these effective concentrations. The close correspondence also suggests a single site of action for DNP.

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Received June 23, 1952. P.S.E.B.M., 1952, v81.

Chemotherapy of Experimental Tuberculosis with Quinoline Derivatives. (19779)

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New derivatives of aminoquinolines were investigated for tuberculostatic action and for therapeutic effect on tuberculosis in guinea pigs. Derivatives of 3-aminoquinoline were emphasized, as this compound is highly bacteriostatic. In the present experiments a number of allied compounds were also tested, including hydrazine derivatives of quinoline and naphthylene as well as some 2-substituted quinolines. A few previously reported compounds were included for comparison.

Methods. The bacteriostatic studies were performed on virulent tubercle bacilli, strain No. H37Rv in Kirshner's synthetic asparagin